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PROGRESS REPORT

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NORTHERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION

AGRICULTURAL RESEARCH SERVICE

This progress report includes a summary of the current research of the Division and a preliminary report of progress made during the preceding year. It is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1968, and March 31, 1969. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois.

UNITED STATES DEPARTMENT OF AGRICULTURE

Washington, D. C.

July 1, 1969

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INTRODUCTION

The Northern Utilization Research and Development Division, located at Peoria, Illinois, is one of five research divisions of the Agricultural Research Service concerned with the development of basic knowledge of chemical composition and physical properties of farm commodities and with the application of this knowledge to the development of new or improved products and processing technology that will enhance utilization of these commodities. The other Utilization Research and Development Divisions are the Eastern at Philadelphia, Pennsylvania, the Southern at New Orleans, Louisiana, the Southeastern at Athens, Georgia, and the Western at Albany, California.

The need and importance of utilization research on farm commodities arise from the fact that the farmer is not organized to carry on modern scientific research to maintain old, and create new, markets for his products. The Northern Division is responsible for the Department's utilization research on corn, grain sorghum, soybeans, flax, crambe, and new crops. Its research on wheat emphasizes industrial utilization and milling technology, and that on forages is limited to a search for the cause of toxicity occasionally displayed by tall fescue grass. Responsibility for research on food and feed utilization of wheat and for the Department's primary utilization research program on forages is assigned to the Western Division.

The scientific research effort at the Northern Division amounts to approximately 195 scientist man-years. In addition, the Division supervises domestic research contracts equivalent to 12.7 scientist man-years and grants equivalent to 16.5 scientist man-years, and sponsors a comprehensive program of research comprising 23 PL 480 grants to foreign institutions.

Following are some of the recent utilization research accomplishments of the Northern Division.

Starch reinforced rubbers for durable, colorful products. In studies to increase industrial uses for cereal starches, Department scientists have developed three starch-based reinforcing agents for rubber (zinc starch xanthate, starch xanthide, and starch-resorcinol-formaldehyde resin). Laboratory-scale evaluations show that these starch products are comparable to medium-grade carbon black and superior to common clays in improving tensile strength and most other physical properties of natural and synthetic rubbers. All three of the starch products are incorporated into rubber by minor variations of a simple basic process which is compatible with current practices in the industry.

Masterbatches of selected starch-reinforced rubbers were prepared and evaluated for a number of applications under a research contract by The Institute of Polymer Science, University of Akron. On the basis of cost and overall properties, the starch-reinforced rubbers are competitive with conventional rubbers for a number of applications and superior in others. The best opportunities for use of starch-reinforced rubbers are in the production

of general-purpose molding compounds, shoe soling, and oil-resistant rubber products. Starch-reinforced rubbers are naturally semitransparent, which allows them to be dyed or pigmented to give brightly colored products.

One basic patent covering starch xanthate-reinforced rubbers has been granted. A second patent has been allowed (but not yet issued) on the use of starch plus a small amount of resorcinol-formaldehyde resin as a reinforcing agent. An application is being filed covering process improvements which permit the use of larger amounts of starch with increased strength properties of vulcanizates. Current annual domestic consumption of black and nonblack reinforcing agents in rubber is approximately 5 billion pounds; thus, the potential market for starch-based reinforcing agents is exceedingly large. This new technology has created more domestic and worldwide interest than any other development by the Northern Division over the past several years.

Low-cost high-performance cationic flours for papermaking. In studies to increase the industrial utilization of cereal flours, Department scientists have developed cationic corn, wheat, and sorghum flours for use as internal additives in papermaking. These products in evaluations on a 32-inch experimental paper machine are as effective in improving strength of paper and in increasing retention of fillers and pulp fines as the best quality commercial cationic starches. Moreover, the cationic flours are considerably less expensive.

They are prepared by treating dry flour first with ethylenimine, then with acid. Since a dry process is used, recovery is quantitative and no waste disposal problems are encountered like those associated with the production of conventional cationic starches, which are made by wet methods.

The laboratory-scale process for making cationic flours was readily scaled up in the pilot plant to provide products in quantities sufficient for several projected trials in commercial paper mills. One such trial with cationic corn flour has been made. The results were as good as currently obtained in the mill with equal amounts of a commercial product costing twice as much as the projected selling price of 12 cents per pound for cationic corn flour. Representatives of the dry corn-milling company who arranged for the paper mill trial were sufficiently encouraged by the results to indicate that they would continue with their plans for industrial development of our product. Two other dry millers are also cooperating with us in the evaluation and promotion of cationic flours.

One of the dry millers, from a survey of the paper industry, has estimated that the potential market for cationic flours in papermaking is around 200 million pounds per year. A large part of this will be new markets made possible by the low-cost and high-performance characteristics of cationic flours.

High-amylose starch use--advanced by new analytical technology. Department scientists have developed new and improved analytical and control technology for high-amylose corn that is aiding in the accelerated production and utilization of this increasingly important U.S. crop. Increased efficiency

and speed of analysis have provided information and procedures for guiding the selection of varieties for planting and evaluation of the materials produced.

Over 1 million bushels of high-amylose corn were grown under contract (for a premium) in 1968 on over 10,000 acres of land. The resultant corn was milled by five industrial companies into the desired finished products. Present production is increasing at a rate in line with the high-amylose seed that can be developed and made available.

High-amylose starch is finding new and growing markets in such uses as glass-fiber size, paper coatings and additives; water-soluble and edible films; and as a candy (gum drops) ingredient. The farmer, industry, and consumer are all benefiting from this development through increased profits, lower costs, and improved products.

Instant CSM food blend for domestic and foreign food aid programs. A nutritious food blend for worldwide feeding, which is especially suited as a high-protein supplement for weaning infants and for small children, has been developed by USDA engineers. Called "Instant CSM" (Corn-Soy-Milk), it is similar in composition to the regular CSM that has been distributed abroad in amount of nearly 1 billion pounds by voluntary relief agencies, but it is completely precooked and ready for instant consumption by merely mixing with water and allowing to stand for a few minutes. Elimination of cooking is important because of fuel shortages and costs in many areas. It also contains sugar and more salt to increase its acceptance by children. The corn and soy components are individually cooked to the optimum degree for each, then dry-blended with nonfat dry milk, vitamins, sugar, salt, and minerals. The resultant blend serves as a palatable, acceptable, and nutritious food that offers great potential in world feeding. As a direct result of this work, multi-ton quantities of the product have been prepared for UNICEF at two corn-processing plants in the United States and distributed by that agency to East and West Africa and to India, where acceptance has been very favorable. Commercial corn processors in the U.S. can now produce this material at only a very slight increase in processing cost, in addition to the higher cost of the added sugar. This new product could be distributed in place of, or in addition to, regular CSM in the foreign donations program, and should also find a place in our domestic donations program. Approximately 300 million pounds of the regular CSM were purchased by Commodity Credit Corporation from U.S. companies during the past year for foreign donations.

New high-yielding source of L-asparaginase. Survey of many strains of a variety of microorganisms in the ARS Culture Collection at the Northern Laboratory has led to the discovery of a microorganism, Erwinia aroideae, which produces three times more L-asparaginase than reported from other sources. Recently, L-asparaginase was shown to be remarkably effective against one form of cancer, leukemia. L-asparaginase is an enzyme which destroys the amino acid, asparagine, without which the cancerous cells cannot live; normal body cells do not require this amino acid. Up to now, the source of L-asparaginase was the bacterium, Escherichia coli, and yields were limited. Besides giving higher yields of L-asparaginase, E. aroideae

is also superior to the other sources of the enzyme because it produces only L-asparaginase and not other forms of the enzyme. A successful fermentation was developed to produce this enzyme based on a glucose yeast extract medium. Because the NU enzyme is immunologically distinct, it can be used in patients who become allergic to the E. coli during chemotherapy. Animal tests indicate the new enzyme source is effective in reducing leukemia in experimental animals. An application for a public-held patent has been applied for.

Corn protein discoveries are important keys to better nutrition and grain processing. Discoveries in USDA research on corn protein structure and location in grain cells help explain differences in nutritional value and processing behavior of different corn varieties. The new findings resulted from advances in methods of protein research and will benefit corn breeders and processors.

By separating various microscopic structures found in corn grain cells, it was established that individual protein types were located in different cell components. Zein, the least nutritious protein, is located in small protein bodies. The smaller amounts and size of zein-containing protein bodies in high-lysine varieties were demonstrated in these studies. Since the total protein content is fairly constant, the reduction in zein results in an increase in the preferentially desirable glutelin which forms a fibrous matrix that binds cell constituents.

The insolubility of glutelin protein has been found to be due to the fact that its chains are linked together by sulfur bonds. This linkage is responsible for the poor dough quality of corn flour. The linkage was shown to be broken by chemical treatments prior to grain processing for starch production. Now genetic selection or new chemical treatments may be sought to advance corn uses.

USDA research opens military market for soybean salad oil. Based on results of stability tests conducted by USDA scientists, the U.S. Army Natick Laboratories are now drafting a new proposed section for the salad oil specification to include a soybean oil with a maximum linolenic content of 3 percent and packaged under nitrogen.

Most salad oil for the Armed Forces has been purchased under specifications that did not permit over 0.5 percent linolenic acid in the oil. This requirement was established because the Armed Forces need salad oils that are stable in their original packages for 6 months at 100° F. Early tests found linolenic-containing oils to have insufficient stability. For example, as formerly processed and packaged, oils like soybean salad oil with 6 to 9 percent linolenic lost their initially acceptable flavor in 3 to 6 weeks at 100° F.

By 1967, some domestic suppliers of soybean salad oil had improved the stability of their product by procedures which followed recommendations of our Northern Division. Consequently, USDA scientists undertook new studies on the stability of these improved commercial soybean salad oils in cooperation with the Natick Laboratories of the Department of the Army. These studies

demonstrated that some of these commercially available, specially processed soybean salad oils maintained oxidative stability and good flavor quality on prolonged storage of 9 to 12 months at 100° F. These studies also enabled USDA to provide the technical details of composition, processing, and packaging required for preparation of a military specification. Since the Armed Forces are reported to use 35 to 45 million pounds of salad oil each year, this new specification provides a significant new market for soybean salad oil. It will also give the military greater freedom in procurement.

New plant substance with anti-leukemia activity. A new compound that slows the progress of experimental leukemia in laboratory mice has been isolated by USDA scientists from a yew-like evergreen shrub. The substance is effective at very low dosages, considerably below the level at which it is toxic to the animals. Under preferred conditions of administering the drug, treated leukemic mice lived more than twice as long as untreated ones. Though the mice were not cured, their lengthened survival in the test is useful in predicting beneficial effects in treating leukemia and possibly some solid tumors in other species including man. The results are sufficiently encouraging to warrant preclinical pharmacological investigations in additional animals such as dogs and primates. The USDA chemists cooperated in this research with scientists of the Cancer Chemotherapy National Service Center of the Department of Health, Education, and Welfare, who directed animal tests and evaluations.

This anti-leukemia factor was discovered as a result of an extensive chemical survey of the plant kingdom to find useful new industrial, food, feed, and pharmaceutical substances in plants that can be grown as new crops in the United States. Altogether more than 10,000 plant samples have been studied and about 200 of these have been tested for the presence of anti-tumor substances.

CORN UTILIZATION

USDA and Cooperative Programs

Problem Area		Scientist Man-Years FY 1969 (Estimated)		
No.	Title and Activity	Intra- mural	Extra- mural	Total
406:	New and Improved Food Products from	:	:	:
:	Field Crops	:	:	:
:	Chemical Composition and Physical	:	:	:
:	Properties	7.3	1.1	8.4
:	Color, Texture and Other Quality Factors	7.1	:	7.1
:	Microbiology and Toxicology	4.1	1.7	5.8
:	Technology--Process and Product	:	:	:
:	Development	3.6	:	3.6
:	Subtotal	22.1	2.8	24.9
407:	New and Improved Feed, Textile and	:	:	:
:	Industrial Products from Field Crops	:	:	:
:	<u>Industrial Products</u>	:	:	:
:	Chemical Composition, Physical	:	:	:
:	Properties and Structure	8.0	1.1	9.1
:	Chemical and Physical Investigations	:	:	:
:	to Improve Products	10.0	3.0	13.0
:	Microbiology and Fermentation	13.1	0.7	13.8
:	Technology--Process and Product	:	:	:
:	Development	6.1	3.5	9.6
:	Subtotal	37.2	8.3	45.5
702:	Protect Food Supplies from	:	:	:
:	Harmful Microorganisms and	:	:	:
:	Naturally Occurring Toxins	:	:	:
:	Microbiology and Toxicology	6.0	0.4	6.4
:	Subtotal	6.0	0.4	6.4
901:	Alleviate Soil, Water, and Air Pollution	:	:	:
:	Chemical and Physical Investigations	:	:	:
:	to Improve Products	1.7	0.4	2.1
:	Subtotal	1.7	0.4	2.1
:	Total	67.0	11.9	78.9

Domestic program supplemented by PL 480 funds in 8 countries totaling 669,884 U.S. dollars equivalent (Austria, Belgium, India, Japan, Netherlands, Poland, Spain, United Kingdom).

Problems and Objectives

The farmer sells 100 million bu. of grain to make 3 billion pounds of cereal starches and flours that are used annually in the U.S. for industrial purposes. Corn is the source of most of these products. These outlets are constantly threatened by synthetic products derived from nonagricultural sources. Increasing the present and future competitive position of corn starch and flour in industrial markets requires development of new and improved products that will do a better job in outlets such as the paper industry, industrial chemicals, adhesives, protective coatings, plastics, elastomers, and thickening agents. Almost 80 percent of the U.S. annual production of corn is used as animal feed. Because of the extremely large volume of this outlet, even small improvements in quality or processing efficiency give direct gains to industry and to the farmer. Utilization of corn in foods also is an outlet of great economic importance. Significant benefits can be realized if we can make cheaper and more nutritious products, including foods from new corn varieties having higher nutritional value than does ordinary dent corn.

Major objectives of the current research are to develop and evaluate alternate ways to:

1. Discover new technology for converting starch by chemical or fermentative means to products for use in the paper, chemical, and other industries.
2. Further the development and industrial utilization of new classes of high-amylose corn.
3. Improve yields and quality of corn dry-milling fractions.
4. Lower the cost of converting corn to more acceptable, varied, and nutritious foods.
5. Remove mold toxins or harmful microorganisms from corn and corn products.

Progress - USDA and Cooperative Programs

RPA 406 - NEW AND IMPROVED FOOD PRODUCTS FROM FIELD CROPS

A. Chemical Composition and Physical Properties

1. Corn proteins. Advances were made in isolation and characterization of proteins in normal and high-lysine corn, in nutritional evaluation of corn milling products, and in processes for improved feed and food products. Sub-cellular particulates of immature high-lysine corns were separated and differences detected in rate of synthesis and type of proteins deposited compared to normal corn. Glutelin protein was separated from mature endosperms and differences were observed between glutelins in normal and

high-lysine grains. Gel filtration chromatography of alkylated-reduced glutelin separated different molecular species. During wet milling of corn, glutelin disulfide bonds are cleaved and protein is partially solubilized by SO₂ steeping. Amino acid analyses, available lysine, biological value, and economic estimation of hominy feed components establish that these dry-mill products are valuable feed supplements for nonruminants. High-lysine corn meal was added to leavened baked goods. Starch-protein separation by air classification of normal and high-lysine flour was improved after treating meal with isotonic buffer.

In the final phases of grant research at Purdue University, Lafayette, Indiana, emphasis has been placed on ascertaining differences between the proteins of normal and high-lysine corns to account for the nutritional advantages of the latter grain. Separations of proteins from both normal and high-lysine corn were investigated by chromatography on Sephadex columns. A rapid procedure for estimating lysine levels in corn grain was tested for utility in screening corn produced in breeding experiments. A wide range of protein and lysine content was observed in 28 opaque-2 hybrids produced by crossing 8 opaque-2 inbreds. Maximum protein was 13.5 percent and lysine 5.4 grams per gram protein. The free amino acid in the grain at various periods following pollination was consistently higher in high-lysine corn than in normal corn. In normal corn the level dropped more than it did in high lysine as maturity was reached.

2. Amino acid analyses. Maximum analytical capability for amino acid determination has been increased to 16 analyses per day with the installation of a TSM amino acid analyzer. This increased capability has allowed increased service and cooperative work with other projects and laboratories. The first phase of a collaborative study on amino acid analysis of soybean meal in cooperation with the Soybean Research Council, to establish standard procedures for industry, is now completed. Analysis of lysine-infused corn samples has shown infusion to be a feasible method of fortifying whole corn with lysine. The reaction of methyl acrylate with lysine in grain is being pursued to determine its utility as a new available lysine method. Work on the use of pyridylethyl cystine as an internal standard for ion-exchange chromatography of amino acids is complete.

Linear programming studies are now run on our 1130 computer using a program supplied by IBM which gives greater flexibility. Food supplement formulation using parametric linear programming has continued and some formulas have been analyzed for taste, odor, and protein efficiency ratio. The advantages of hominy feed as a feed ingredient were studied by parametric linear programming.

In contract research at North Star Research and Development Institute, Minneapolis, Minnesota, research is underway to find ways of estimating the nutritionally essential amino acids, lysine, methionine, threonine, and tryptophan in cereal grains without hydrolysis of the protein. Requirements for such a procedure are to bring all the protein of the grain into solution

and to find a specific reagent for each amino acid to be determined that depends on a characteristic functional group that is free in the unhydrolyzed protein. Conditions were found quantitatively to form the dinitrophenyl (DNP) derivative with the amino group on the ϵ carbon of lysine in unhydrolyzed protein and to prevent the interference of similar DNP products from the remaining amino acids in the protein. The DNP derivative, a condensation product with dinitrobenzene sulfonic acid (DNBS), was estimated from a characteristic high absorptivity at 360 m μ . The method gave accurate analyses for lysine content when applied to seven commercially available isolated proteins. Methods of solubilizing over 96 percent of the protein in Durham wheat flour and in corn endosperm were developed.

3. Structural and chemical differences among corn genotypes. A procedure was developed for examination of subcellular endosperm protein structure in maize. Examination of the subcellular structure and composition of the proteins is useful in classifying various types of corn, in identifying corn with unusual protein characteristic such as high-lysine mutants, and in identifying dry-milled particles. Protein in a corn inbred, B-57, was shown by this technique to be deficient in zein bodies indicating that the protein is of higher lysine content than in ordinary corn. Corn with more than one layer of aleurone cells was found by chance in crosses involving the high-amylose gene and various exotic strains. The aleurone is a concentrated source of high-quality protein and other nutrients. Origin of the aleurone increase was subsequently traced to a South American exotic used in the early crosses. When followed through the F₂ generation, the modified aleurone character appeared to be dominant.

4. Phytin in corn. Research results from studies under a grant to the University of Missouri, Columbia, Missouri, indicate that the corn germ contains nearly all of the phytate and by far the highest concentration of the trace elements in the corn kernel. Heating phytate (pH 5-6) or products containing phytate in an autoclave causes the molecule to decompose so that organic phosphorus is no longer precipitated by ferric ion. Phytate in corn germ is relatively stable with considerably less stability in sesame and soybean. The extractability of phytate varies widely from product to product. A large proportion of the phytate in corn germ can be extracted with water.

5. Corn lipids. Crude fat or oil (nonpolar lipids) was extracted with hexane from whole yellow dent corns and hand-dissected fractions. Most of the nonpolar lipids were located in the germ. Floury endosperm contained approximately 3 percent nonpolar lipids compared to 0.5 percent in horny endosperm. Triglycerides accounted for the major portion of the nonpolar lipids in the whole corns and their hand-dissected fractions. Smaller amounts of hydrocarbons, sterol esters, fatty acids, diglycerides, monoglycerides, phospholipids, and two unidentified components were also present. Separation of triglycerides by gas-liquid chromatography indicated that the whole corns and their hand-dissected fractions were remarkably similar in triglyceride composition averaging as: C50, 4 percent; C52, 30 percent;

C₅₄, 65 percent; and C₅₆, 1 percent. Peroxidase measurements were used to indicate enzyme activity in processed corn fractions. Peroxidase activity in the germ fraction was greater than in the endosperm or pericarp fractions. The degree of enzyme inactivation in roll- and extrusion-cooked grits depended on the amount of heat and moisture used in processing. The enzyme, linoleate hydroperoxide isomerase, isolated from corn germ, was purified and characterized. Two products resulting from isomerase activity were isolated and identified as fatty acids with the general formulae cis-R'-CHOH-COCH₂CH=CH-P and trans-R'-CO-CH=CH-CHOH-R.

6. Reactions of maltose and glucose. Fusion phase diagrams showed the existence of 4:1, 2:1, and 1:1 molecular complexes of maltose with urea; the latter two complexes were isolated. Solvolyses of β -chloro-D-glucose and -maltose in pyridine and dimethylformamide showed new, higher yielding preparative routes to C2-hydroxyl-free α -anomers of tetra-O-acetyl-D-glucose and hepta-O-acetyl maltose. Ethyl 1-thio- β -maltose (70%) was synthesized from maltose octaacetate for the first time; treatment with bromine in methanol gave methyl α -maltoside of 87 percent purity in 81 percent yield. Kinetic studies of the reactivities of the C2 and C3 hydroxyl groups in 4,6-benzylidene methyl α - and β -D-glucopyranosides (and of the C2 and C3 mono-O-acetyl derivatives of these) showed differing hydroxyl group reactivities toward different acetylating agents in pyridine: toward acetic anhydride, the C3-OH was more reactive; toward acetyl chloride, the C2-OH was more reactive. 1,2-O-Ethylidene acetals of D-glucose and maltose were synthesized by borohydride reduction of the 1,2-orthoacetate acetoxonium ions. The ratio of endo to exo substituent isolated was shown to vary with the solvent medium used in the reduction. NMR analyses showed varying conformations of the pyranose ring depending upon the steric crowding provided by bulky endo substituents on the 1,3-dioxolane ring of different acetal or ketal derivatives.

B. Color, Texture and Other Quality Factors

1. Browning reactions. Study of the lowly volatile browning reaction products obtained by pyrolysis of 1-deoxy-1-piperidino-D-fructose at 106° C./1 mm. and separated by gas-liquid chromatography was concluded with the identification of 15 compounds by IR, NMR, and mass spectrometry, and by synthesis. Seven of the compounds were piperidine amides or salts of carbonic, formic, acetic, glycolic, lactic, and butyric acids. The others were α -piperidino- γ -butyrolactone, four furan derivatives, two piperidino 4-carbon reductones, and piperidino-hexose-reductone. The hexose is split mainly into 2- and 4-carbon fragments. Similar pyrolysis of 1-deoxy-1-prolino-D-fructose at 135° C./1 mm. gave corny, bready, and caramel-like aroma compounds. Two fragrant caramel aroma compounds were identified as diacetyl-formoin and 4-hydroxy-2,5-dimethyl-3(2H)-furanone. Present in much larger amount was 5,6-dihydro-3,5-dihydroxy-2-methyl-1,4-pyrone, a new browning reaction intermediate. Two caramel-flavored dihydrofuranones, isolated recently by Tonsbeek et al. from beef broth, were synthesized in an unequivocal way from isopropylidene derivatives of D-fructose and D-xylose, and from model sugar-amine browning reactions.

C. Microbiology and Toxicology

1. Studies on enzymes. Glucoamylase has been immobilized successfully by two methods. In the first method it has been bound to DEAE cellulose in low ionic strength buffers where it shows about 30 percent of the expected activity. Columns of DEAE cellulose-enzyme complex are capable of continuously converting starch to glucose over extended periods of time. In the second method glucoamylase was covalently linked to s-dichlorotriazinyl cellulose to form an enzyme cellulose complex. This complex was stable and was able to convert a dilute solution of a soluble dextrin to glucose in about an 85-percent yield in a continuous stirred vessel system. Conditions for the production of 3-ketomaltose from maltose by Agrobacterium tumefaciens B-36 were determined. Initial maltose concentrations were found to be directly proportioned to the time at which 3-ketomaltose started to accumulate in the medium. However, higher levels of maltose, though delaying the onset of 3-ketomaltose formation, prolong the peak production period. About 25 percent of the maltose is converted to 3-ketomaltose.

Enzyme preparations, obtained from culture filtrates of Rhizopus oligosporus by ammonium sulfate fractionation and gel filtration, were evaluated for cheesemaking. After aging for 3 months, the cheese developed an undesirable bitter taste, although its body and texture were comparable to the ones made with animal rennin. The enzyme preparation can be separated into several components having different ratios of milk-clotting and casein digestion activities. The multiple forms of the enzyme produced by the mold were further shown to exist naturally and they are not an artifact of purification, or products of self-digestion. The antibacterial agent produced by R. oligosporus can be successfully separated from the proteolytic enzyme by gel filtration. The compound is active against many gram-positive bacteria, especially those growing under microaerophilic or anaerobic conditions. The discovery of the antibacterial agent produced by the mold offers a clearer understanding of the nutritional value of tempeh in the diet of Indonesians; it is well established that antibiotics, in addition to minimizing infections, elicit growth-stimulating effects in animals especially when diets are nutritionally inadequate.

In studies under a grant to the University of Nebraska, Lincoln, Nebraska, it has been determined that the major difference between the two isozymes comprising the glucoamylase from Aspergillus niger is the length of their protein chains. Content and distribution of carbohydrate in the two isozymes are similar: heterogeneous di- and trisaccharides are attached glycosidically to serine and threonine in the protein backbone. Analogous results were obtained for the glucoamylase from Aspergillus phoenicis.

Under a grant to the University of Arkansas, Fayetteville, Arkansas, a method has been worked out whereby the ratio of products formed by Bacillus subtilis α -amylase can be used to determine the nature of the enzyme binding sites. The unitary free energy of each binding site was calculated using site I as the reference and setting it equal to zero. The catalytic site was

found to be between binding sites III and IV and must always be occupied to get cleavage. Sites I, II, and V through IX all release energy upon association with the substrate. Therefore, it can be concluded that the overall binding site of B. subtilis α -amylase spans nine monomer units. Lysozyme has been radioactively tagged with cello-oligosaccharides. These enzyme-substrate complexes are stable and can be used to study conformational changes taking place during enzyme binding. An ultramicro method for determination of glucose has been developed which is about 20 times more sensitive than published procedures for this sugar.

2. Taxonomic and related investigations. Recent additions of Mucor pusillus and M. miehei have increased their numbers in the ARS Culture Collection to 59 and 10 strains, respectively. Under optimum conditions, 55 of the M. pusillus strains produced zygosporangia when mated. NRRL 1426 which is an old, widely distributed strain of M. pusillus is included in those giving zygosporangia. Examination of the sporangiospore topography of 22 species and 5 varieties of Rhizopus with the scanning electron microscope has led to the discovery of apparently four general types of sporangiosporangia in the genus.

Due to difficulties encountered in devising techniques under a grant to the University of Minnesota, Minneapolis, Minnesota, no significant data has yet been obtained. This project entails developing entirely new methods to relate cell differentiation to biochemical activity of fungal hyphae. Preliminary results do indicate that there are definitely differences in cellular composition and enzyme production from one hyphae segment to another.

Under a grant to Iowa State University, Ames, Iowa, attempts to transform DNA containing the structural gene for amylase from several different organisms to competent doubly mutated and derepressed Bacillus subtilis strains were not successful. The B. subtilis mutant was apparently affected only at the amylase control level and not at the amylase structural gene level. Indirect evidence was found that showed that a new amylase was introduced in a singly mutated strain of B. subtilis which was amylase negative but still protease positive. DNA from B. macerans was introduced into the mutant and an amylase-positive culture was produced. The amylase was, however, subject to proteolysis by the protease, showing that it was different than the amylase of the parent B. subtilis strain which was not subject to proteolysis by the protease. The amylase produced by Thermoactinomyces vulgaris is of a multiple nature in that up to four isozymes have been found. All four show the same action pattern on starch with maltose and maltotriose being the principal oligosaccharides found.

In preliminary grant research at the University of Wisconsin, Madison, Wisconsin, the principal investigator has selected for study Aspergillus oryzae var. effusus and Rhizopus oligosporus as his cultures for the investigation. The initial report describes the development of methods of handling these species to obtain biochemical mutants. Basic studies have begun on determining the nuclear condition in both fungi, one of which is reported

to be binucleate and the second multinucleate. Some biochemical mutants of A. oryzae var. effusus have been made, but these have caused unexpected problems because of their very rapid growth and vigor.

D. Technology--Process and Product Development

1. Corn dry milling. During dry milling of artificially dried corn, level and distribution of moisture in degerminator stock affected both yield and quality of prime goods (table grits, meal, and flour) recovered by laboratory roller milling and grading of the stock. As moisture content of stock at start of individual milling runs was increased from 10 to 20 percent, yield of prime goods decreased slowly and their fat content also decreased, first rapidly, then moderately, and finally slowly or not at all (fat content under 1 percent desired). Ash, protein, and crude fiber contents of prime goods also followed a pattern somewhat like that for fat content. However, fat along with yield of prime goods is considered the best index for determining proper moisture level of degerminator stock for the roller milling operation. Overall, a milling moisture level of 16 to 16-1/2 percent (based on analytical method employed) proved most satisfactory for the corn processed. Milling on same day as degermination was better than delayed milling. Tests on cold tempering and degermination of high-lysine corn showed the tempering conditions employed had little effect on yield or on reduction of fat content of the prime goods. Yield from high-lysine corn was lower and fat content higher than for prime goods from normal dent corn.

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL PRODUCTS FROM FIELD CROPS

Feed

A. Microbiology and Toxicology

1. Beta carotene synthesis. Work continued under a PL 480 grant to the Agricultural University, Poznan, Poland, on enhancement of carotenogenesis in mated cultures of Blakeslea trispora by addition of spent mycelium of various molds (including B. trispora) and yeasts. Mycelia varying in age from 2 to 10 days were tested. An increase in yield was obtained over the control from mycelial addition, but yields were never as high as when citrus molasses was used as the adjuvant.

Isonicotinic hydrazide (INH) was found to stimulate carotenogenesis when added at concentrations of from 0.01 to 0.02 percent; higher concentrations were inhibitory to β -carotene production. A maximum yield of 167 mg./100 ml. was attained on INH addition (control with no adjuvant 38 mg./100 ml.; control with 5 percent citrus molasses added, 124 mg. percent). Addition of INH to the medium after 48 hours of fermentation was not as effective as addition at zero hour. INH could not replace molasses, but was complementary to it.

Difficulty was encountered with culture degeneration. New cultures have been supplied.

Industrial Products

A. Chemical Composition, Physical Properties and Structure

1. Physical and chemical studies on starch components. Optical rotation studies in the solvent systems H₂O-DMSO and formamide-DMSO show a discontinuity in specific rotation for solutions of some α -1,4-linked, open-chain polymers such as amylose. The discontinuity occurs in a region of solvent composition where the solvent components show evidence of hydrogen bonding with each other. A simple solvent effect cannot cause the specific rotation discontinuity, because solutions of dextran, α -Schardinger dextrin, and methyl- β -maltoside do not show a discontinuity. Hydrodynamic studies show the overall behavior of amylose is random coil-like in H₂O, DMSO, and formamide. Thus, we postulate that amylose undergoes a symmetry change in the mixed solvent systems that is caused by a change in net helical twist to the backbone or by a destruction of amylose-amylose chain interactions by a good solvent.

Amylose complexed with ethylene diamine forms a pliable film that seems to be more stable under atmospheric conditions than an amylose-DMSO film.

Structural and conformational studies with NMR have proved invaluable in promoting research in other areas of research. Some new δ -3 and new epoxy acids were characterized. Structural conformations were determined for methyl-9(3,4-methylene dioxyphenyl)-formyl-nonene-8-oate and methyl-9(3,4-methylene dioxyphenyl)-8-formyl-9-hydroxynonate. Structural studies on egyptol are presently in progress.

Under a grant to Arizona State University, Tempe, Arizona, studies based on literature data and molecular space filling models of amylose indicate: (a) two chain arrangements fit the fiber repeat distance of 22.6 Å in KOH-amylose; (b) besides the regular left-handed helix with sixfold screw axis, a non-helical left-handed chain is possible in KOH-amylose; (c) nonhelical chain linkages between residues 1 and 2, 2 and 3, 4 and 5, and 5 and 6 are similar to those found in V amylose; while linkages between residues 3 and 4 and 6 and 1 are of the type found in KBr-amylose; and (d) the three-dimensional Patterson map favors the irregular chain structure.

Possibilities of forming V_{DMSO}-type films having the ability to form oriented crystalline regions during the process of film preparation are being examined. Preliminary X-ray examination of an amylose complex precipitated from solution at high pH shows crystalline KOH-amylose in the product and may be interpreted as evidence of an extended amylose conformation in strongly basic solution.

In studies under a grant to the State University of New York, Syracuse, New York, quantitative evaluation of laser generated light-scattering patterns from single starch granules is, in general, in good agreement with theoretical predictions. Discrepancies between theory and measured relative intensities of higher order maxima are believed to be caused by the presence

of unordered material in the hilum region of the starch granule. Comparison of granule sizes determined from single granule light scattering and microscopic measurements show excellent agreement. Hence, previously observed differences between sizes determined by light scattering and microscope observation is caused by polydispersity and shows a dependence of birefringence on granule size. Electron micrographs of carbon surface replicas of platinum-carbon shadowed amylose films show the presence of both rod-like aggregates and some "spherulitic" organization of rod-like elements. Conformational analysis of B-amylose, which includes possible hydrogen bonding terms between all oxygen atoms of a B-amylose chain, leads to a prediction that a sixfold, left-handed helix is the most stable conformation. Evidence of this structure is found in the form of a sixth order meridional spacing on the X-ray pattern from an oriented B-amylose fiber.

In grant research at Southern Illinois University, Carbondale, Illinois, attempts to detect bromine free radicals in chloroform solutions of bromine irradiated with either a mercury vapor lamp or an ultraviolet lamp were unsuccessful. No free radicals were detected by EPR in the reaction system involving benzyl glucoside and bromine in chloroform or benzene, although the color of bromine disappeared and a precipitate (assumed to be the α -bromo product) formed. Addition of aqueous sodium carbonate to the reaction mixture and subsequent analysis by TLC showed that glucose and unreacted benzyl α -D-glucopyranoside were present. The presence of glucose indicates that some of the α -bromo product was formed and subsequently converted to glucose via the hemiacetal produced by hydrolysis. Corresponding results were obtained with methyl 2,3,6-tri-O-benzyl- α -D-glucopyranoside. Free radicals, which must be present during bromination, were not detected.

In research conducted under a PL 480 grant to the University of Osaka Prefecture, Sakai, Japan, a polarographic method was developed for the determination of carbonyl groups in starch derivatives, based on reaction of carbohydrates with o-phenylenediamine to give polarographically active quinoxaline derivatives. Derivatization is achieved in 20 minutes. The method is applicable to a wide variety of starch-derived materials ranging in size from the sugars, glucose and maltose, to the high molecular weight polymers, amylose and amylopectin. Results obtained by this method over a wide range of carbonyl concentrations agreed well with those obtained by other conventional methods, each of which was applicable over a part of the range covered by this method. The new method is applicable also to the determination of carbonyl groups in periodate-oxidized starch (dialdehyde starch) at various degrees of oxidation.

Only a small amount of sample, common chemical reagents, simple reaction procedures, short reaction time, and a classical polarographic apparatus are required. The method appears to have promise not only as a practical method for carbonyl groups in starch derivatives, but also as a useful method for obtaining fundamental information on reactions of carbohydrates. This research has been completed.

2. Structure and properties of polysaccharides. Studies on dilute solutions and culture fluids of polysaccharide B-1459 show systems that have high molecular weight, are polydispersible, and are highly concentration dependent. Molecular weights obtained by light scattering on culture fluids show \bar{M}_w values of 15×10^6 to 50×10^6 . A solution preparation by heating lyophilized polymer in 4M urea shows \bar{M}_w of about 1×10^6 as determined by light scattering and sedimentation-diffusion measurements. Measurements in 4M urea solution show that: $(dn/dc)_{546} = 0.135 \text{ ml./g.}$, $\frac{1}{c} \eta_{sp} = 0 = 29 \times 10^2 \text{ ml./g.}$, $\bar{V}_{25} = 0.59 \text{ ml./g.}$, $D_{25}^0 = (2.36 \pm 0.4) \times 10^{-8}, \text{ cm.}^2/\text{sec.}$, and $S_{25}^0 = (5.0 \pm 0.2) \times 10^{-13}$. Viscosity measurements on dispersions were made in a Zimm viscometer in order to be in a Newtonian flow region. Dispersion viscosity in 4M urea and 0.01M ammonium acetate is time dependent and is affected by dehydration and alcohol precipitation of the polysaccharide. Intrinsic viscosity value from $1.5 \times 10^2 \text{ ml./g.}$ to $9.2 \times 10^2 \text{ ml./g.}$ were obtained.

In grant research at East State Texas University, Commerce, Texas, factors influencing controlled introduction of certain substituents (trityl, carboxymethyl, and sulfate) into dextran (M Wt. 65,000) have been established and the corresponding products prepared. A new practical method for determining degree of tritylation (by ultraviolet absorption) has been shown feasible. Application of periodate oxidation to location of sulfate substituents has revealed inhibition of oxidation by the ionic groups to a degree heretofore unreported.

Under the grant at the University of Wisconsin, Madison, Wisconsin, determination of the location of the O-acetyl substituents in polysaccharide B-1973 has advanced to the latter stages of the investigation. A satisfactory method has been devised for blocking the free hydroxyls with carbanilylate groups in the presence of acetyl esters. Hydrolysis of the esters without concomitant migration or loss of the blocking groups has been achieved with dry hydrogen chloride in 2-methoxyethanol and is accompanied by complete depolymerization of the polysaccharide. The partially carbanilylated sugar glycosides resulting from depolymerization have been separated according to their degree of substitution and have been partially characterized. Removal of the carbanilylate groups on the monosaccharides is accomplished more readily with refluxing sodium methoxide than with lithium aluminum hydride--the usual procedure.

Isolation of the monosaccharides permits study of the final steps in the analysis: reaction of deesterified hydroxyls with methylvinyl ether, decarbanilylation, methylation of the liberated hydroxyls, and mass spectroscopy to determine the position of the substituents on the different sugars.

Under a PL 480 grant to the National Sugar Institute, Kanpur, India, aldobi- and aldotriuronic acids (as the barium salts) in amounts from milligrams up to 3 to 5 grams are being accumulated by successive batchwise fractionation of partial hydrolyzates from 10 different gums from Indian trees and shrubs. Optimal conditions for partial acid hydrolysis of each gum have been established individually and procedures for fractionation on cellulose columns

have been continuously improved. A total of 7 aldobi- and 5 aldotriuronic acids have been isolated in pure state, and work on 6 others is in initial stages. These substances are composed of various combinations of uronic acids (β -D-glucopyranosyl uronic, 4-O-methyl-D-glucopyranosyl uronic, or α -D-galactopyranosyl uronic) linked to neutral hexoses (D-galactose, D-mannose, or L-rhamnose) at carbons 2, 4, or 6.

B. Chemical and Physical Investigations to Improve Products

1. Physical properties of starch dispersions. At the University of Graz, Graz, Austria, rheological properties of starch pastes have been measured by capillary viscometry at high-shear rates (about $5 \times 10^5 \text{ sec.}^{-1}$) that approach commercial ranges of shear in paper-coating operations. Good transition of low-shear (rotational measuring device) to high-shear data was observed. Coating formulations (from a paper-coating mill) were examined by the high-shear techniques and good positive correlations obtained. This was not true of the low-shear measurements. The development of a measuring system to predict performance of starch adhesives in coating formulations is said to be a practical realization. This study is being conducted under a PL 480 grant.

In the final phase of work at the Slovenian Academy of Sciences and Arts, Ljubljana, Yugoslavia, waxy maize and high-amylose (70% amylose) starches of American origin were modified by a variety of physical and chemical treatments designed to impart, without granule disruption, new and useful paste properties to the starches. Treatments that produced the greatest changes in gelatinization temperature, paste viscosity, setback, solubility, swelling power, and transparency of the starches were the same as those found and reported previously for corn, wheat, and sorghum starches. They were as follows: Soaking in dilute acids or sodium phosphate, heating dry or in aqueous slurries, ultrasonic treatment of aqueous slurries, or a combination of these treatments. Some difficulty was experienced, however, in obtaining reproducible results with waxy maize products. Modifications of high-amylose starch also showed some difference from the other modified starches in that swelling power did not change when paste viscosity was decreased and solubility increased. Selected modified starches from the five different starches investigated were drum-dried. This treatment did not alter the properties of cooked pastes significantly. This work, now completed, was conducted under a PL 480 grant.

2. New derivatives of starch and related carbohydrates. Reaction of bromoacetaldehyde diethyl acetal with D-mannitol gave a 2:1 mixture of cis-1,2:cis-5,6-di-O-bromoethylidene-D-mannitol and cis-1,2:trans-5,6-di-O-bromoethylidene-D-mannitol. Studies established that di-O-bromoethylidene-D-mannitol formation proceeded through a 1,2-O-bromoethylidene-D-mannitol and that monobromoethylidene acetal formation was equilibrium controlled and its conversion to the dibromoethylidene acetal was kinetically controlled and irreversible because of insolubility.

In the above study 2,5-di-O-methyl-D-mannitol was needed for comparison purposes. As present syntheses were lengthy and difficult, a simple two-step method was devised for the preparation of this dimethyl mannitol.

Reactive trans-fused cyclic carbonate and thionocarbonate groups have been introduced into mono- and polysaccharides. Compounds containing carbonyl-seeking groups such as alcohols, thiols, and amines readily form covalently bonded adducts with the carbohydrate carbonates methyl 4,6-O-benzylidene- α -D-glucopyranoside 2,3-carbonate and the 2,3-thionocarbonate. Amino acids react readily at the carbonyl and thiocarbonyl centers to form covalently bonded carbohydrate-amino acid products. Carbonate ring opening of the trans-carbonate by para-substituted benzyl alcohols is first order in both carbonate and alcohol. Procedures have been developed whereby carbohydrates can be derivatized with pendant side chains which contain reactive epoxide and episulfide functional groups.

Sufficient amounts of cationic cereal flours were prepared for evaluation as wet-end additives on the Northern Division's large paper machine in the production of clay-filled bond and bag papers. Corn, wheat, and sorghum flours were allowed to react in a semidry process with 3 wt. percent ethylenimine. Avoidance of water as a reaction medium offers economic advantages. Performance of our cationic flours in paper machine trials was in every respect as good as or better than that of a high-quality commercial cationic starch wet-end paper additive made by conventional wet processes.

In preliminary flocculation tests, cationic wheat flours (3 wt. percent ethylenimine) were found to be considerably better than sorghum or corn flour derivatives or commercial cationic starches. This is the first instance where a preferential benefit has been obtained from a particular cereal flour in a nonfood application.

Wheat and corn flours have been reacted in the dry state with 1 to 5 wt. percent formaldehyde. Values for swelling, dispersibility, and solubility of these flour products in water and in dilute alkali and acid have shown the materials to be crosslinked. Preliminary evaluation tests as yet have not shown any particular advantage for the crosslinked materials over the present flour products.

In research, under grant, at the University of Arizona, Tucson, Arizona, copolymerization of starch, methyl α -D-glucopyranoside, and D-glucose with certain diepoxides was effected. Yields were low and the crosslinked polymers were probably of low molecular weight. A synthesis of p-di(epoxyethyl)benzene and the meta isomer was worked out. Several attempts to obtain the ortho isomer were unsuccessful. Action of trimethylsilyl chloride on an epoxide was found to be a general reaction which gave the related chlorohydrin trimethylsilyl ether.

In grant research at the Ohio State Research Foundation, Columbus, Ohio, starch acetals of wheat and corn starch have been prepared by reaction with

various vinyl ethers. Acetals formed by reaction of vinyl ethers with carbohydrates are subject to decomposition by acid-catalyzed hydrolysis. Some studies on the hydrolytic removal of acetal groups showed good stability of benzylidene groups under some conditions. Also, the tetrahydro-2H-pyran-2-yl-acetals of corn starch were more stable than acetals from reactions with alkyl vinyl ethers. A variety of reactions have been studied for preparing acetals of glucose as model compounds for later adaptation to synthesis of starch acetals.

In other grant research at the Ohio State Research Foundation, studies have been continued on the introduction of amino groups into glucose and extended to the preparation of amino-amylose derivatives. 6-O-Tritylamylose was oxidized with periodic acid to give dialdehyde amylose, approximately 50-percent conversion. The oxidized product was nitromethylated and reduced with lithium aluminum hydride. Though the reduction procedure caused dehydration, a water-soluble, nondialyzable product with one aminated glucose unit per 2 glucose units was obtained in 5-percent yield. Experiments are being continued on the quantitative isolation of amino sugars obtainable on the above aminated amylose. Working with the model compound 4,6-O-benzylidene- α -D-glucopyranoside, two pure crystalline compounds have been isolated after nitromethylation.

Under grant research at Purdue University, Lafayette, Indiana, D-glucopyranose 6-phosphonate was synthesized in low yield to provide another D-glucose derivative containing carbon-bound phosphorus. Synthesis of two new sugar derivatives, 3-amino-3-deoxy-4-thio-D-ribofuranose and 6-amino-6-deoxy-5-thio-D-glucopyranose, with adjacent mercapto and amino groups was accomplished. Aromatization of 4-thio-D-ribofuranose at reflux in dimethylformamide, with mercuric chloride and piperidine as acid-base catalyst, gave a 46-percent yield of 4-acetoxy-2-acetoxymethylthiophene.

Nucleophilic displacement of the 3-O-tosyl group in 1,2:5,6-diisopropylidene-D-glucofuranose with sodium azide or potassium thiolacetate in dimethylformamide gave the corresponding 3-azido or 3-O-thiolacetyl α -D-allofuranose derivatives. Reduction of these derivatives with Raney nickel gave the 3-amino-3-deoxy and 3-deoxy derivatives, respectively. Photolysis of an acetylated methyl α -D-glucopyranoside that contained a 6-azido group resulted in conversion of the azido to an imino group. The acetylated 6-imino methyl glucoside was isolated crystalline in about 30 percent yield.

At Southern Illinois University, Carbondale, Illinois, under a grant, an improved method for preparing alkyl α -D-glucopyranosides was completed. Amylose triacetate or D-glucose pentaacetate was equilibrated with the selected alcohol and a sulfonic acid catalyst at the reflux temperature; the alkyl β -D-glucopyranoside present was hydrolyzed with β -glucosidase; the D-glucose was removed with strongly basic anion-exchange resin; and the alkyl α -D-glucopyranoside was recovered from the effluent as a white, hygroscopic

powder in high yield. 2-Propyl, 2-butyl, 2-methylpropyl, 1-pentyl, and 2,2-dimethylpropyl α -D-glucopyranosides were new among the twelve glycosides prepared.

Additional methyl glycosides of malto-oligosaccharides were synthesized that contained 4-O-ethyl, 4-O-(1-butyl), and 4-deoxy terminal glucose units. The terminal 4-deoxy malto-oligosaccharides were hydrolyzed by β -amylase whereas the 4-O-alkyl ethers were not. Methods developed for this work were extended to prepare 14 methyl 4-O-alkyl-2,3,6-tri-O-benzyl- α - and β -D-glucopyranosides. 4-O-Methyl, -ethyl, -1-propyl, -2-propyl, -1-butyl, -2-butyl, and -1-octyl ethers were prepared in dimethylformamide with the appropriate alkyl halide and sodium hydride. The α - and β -anomers were separated in gram quantities by thin-layer chromatography.

Several routes to deoxygenated derivatives of carbohydrates have been explored under grant research at Ohio State University, Columbus, Ohio. Photolysis of dimethylthiocarbamate derivatives of carbohydrates leads to the corresponding deoxy derivatives. This reaction proceeds via isomerization of the C-O bond to C-S, with subsequent homolysis of the latter to C-H. Photolysis of azide derivatives of primary hydroxyl groups in simple sugars provides a new route to aldehyde derivatives. Extension of the azide reaction to amylose has been successful and affords a polyaldehyde derivative of the starch component. Various addition reactions to unsaturated derivatives of carbohydrates have led to novel deoxygenated derivatives of sugars. Synthesis and reactions of glycosylsulfenyl bromides have been studied in detail.

In studies under a PL 480 grant at the Institute for Fibres and Forest Products Research, Jerusalem, Israel, the viscosity of potassium hydroxide solutions of hypobromite-oxidized potato amylose and waxy-maize, limiting viscosity numbers and Huggins viscosity slope constants were determined both with and without previous treatment with sodium borohydride. Results showed that, with samples untreated with borohydride, a partial degradation occurs immediately on dissolution in potassium hydroxide solution. As a result, limiting viscosity numbers are reduced by a factor of two. This indicates the presence of highly alkali-labile sites in the oxidized starches. It is clear that borohydride-pretreatment of oxystarch is imperative before viscosity determinations in alkali. Considerable changes in slope constant were found with oxidized starches as compared with unoxidized and borohydride-treated samples.

Hypobromite-oxidized amylose was acid-hydrolyzed and anionic products were separated on an ion-exchange resin. Analysis by two-dimensional TLC revealed glucuronolactone and an unidentified keto-acid. These studies were completed during the year.

The Na, K, Li, Mg, and Ba alkoxides of starch prepared by the equilibrium sorption method from activated starch at the Plastics Research Institute TNO, Delft, The Netherlands, were analyzed to determine uniformity of substitution. For this purpose, the metal alkoxides were converted to the methyl

ethers of starch. These were then hydrolyzed to give the corresponding mixtures of glucose and methyl glucoses, and the mixtures were resolved by gas-liquid chromatography. Distribution of unsubstituted and substituted (mono-, di-, and tri-) glucose in the hydrolyzates followed nearly the ideal uniform pattern predicted by statistical theory. The corresponding metal alkoxides of starch, prepared by the classical method of evaporative removal of methanol from suspensions of starch in methanolic solutions of the metal methoxides, had very nonuniform distribution of the metal substituents.

The new grades of metal alkoxides which were developed constitute intermediates for the preparation of ether and other derivatives of starch whose properties may be better suited for certain applications than those of counterparts prepared from classical metal alkoxides of starch. This work is being conducted under a PL 480 grant.

At the Jadavpur University, Calcutta, India, under PL 480 grant, complexes of D-glucose and maltose with calcium salts, calcium hydroxide, and calcium ethoxide were prepared in solution or in crude dry form. The complexes were not stable in aqueous or partially aqueous solvents. Suitable procedures for isolation and analysis of these complexes have not yet been developed.

3. Chemical products from starch. Important variables affecting starch xanthate latex masterbatching and the resulting vulcanizate properties have been evaluated. Commercial unmodified corn starch and yellow corn flour are equally effective reinforcing agents and are superior to lower molecular weight products. Preparation of oil-extended and hydrated-silica-reinforced starch-rubber masterbatches appears feasible. A xanthate D.S. of about 0.12 is optimum. Flexibility in obtaining desired processing and end product requirements is achieved by varying crosslinking agents and conditions. Optimum starch loading for oven-dried masterbatches was about 30 phr. Representative starch-rubber masterbatches received favorable evaluation from a major rubber company. Low-cost improved starch-resin reinforcing agents were prepared from preformed resole resin.

Urethane plastics were prepared from a polymeric diisocyanate and a series of mixed polyols. The polyols used were castor oil and the products from alcoholysis of castor oil with glycol glycosides. In formulating the plastics the equivalent ratio of isocyanate to polyol (NCO/OH) was varied from 1 to 2 and the polyol equivalent weight from 200 to 336. Starch at various levels was incorporated into these same formulations to determine its effect upon the chemical cost and properties of urethane plastics. The plastics were prepared by uniform mixing of the components and subjecting the mixture to pressure-molding at 140° C. As the amount of starch was increased from 0 to 60 percent and the NCO/OH ratio from 1 to 2, and as the polyol equivalent weight was decreased from 336 to 200, there was a corresponding increase in tensile strength, hardness, and heat resistance of the plastics. Cost estimates indicate that the use of starch and starch-derived glycol glycosides can significantly reduce chemical costs of urethane plastics.

Starch xanthide, under optimum conditions of preparation and application developed under contract at the Institute of Paper Chemistry, Appleton, Wisconsin, is a uniquely beneficial papermaking additive. Pulp drainage and both wet and dry strength of paper are improved. Three problem areas are identified in which further work is desirable for improving utilization opportunities: (1) An alternative for use of alum as a retention aid, (2) coloration of paper (not significant in unbleached grades), and (3) development of an applications system "---so that the production of starch xanthide by the papermaker will require the least transfer of technological information."

In research under a grant to Western Michigan University, Kalamazoo, Michigan, optimum conditions for the homogeneous catalytic air oxidation of methyl α -D-glucoside to the glucuronoside are being sought for application to the oxidation of starch to 6-carboxyl starch. Air oxidation of methyl α -D-glucoside in water at pH 9 with a platinum chloride-stannic chloride complex catalyst was slow and after 1 week 50 percent of the starting glucoside was recovered. Use of a potassium ferricyanide-osmium tetroxide catalyst appears to be more promising. Air oxidation of corn starch in water suspension at 40° C. and pH 9 with ammonium vanadate catalyst produced a starch having a high percentage of carbonyl groups with little carboxyl content.

The work on the copolymerization of cereal starches with polysaccharide gums at Ahmedabad Textile Industry's Research Association, Ahmedabad, India, showed that products could be obtained that may have value in specific applications. Evaluation of several of the polymers as deflocculants in paper-making indicated that the products were unsuitable for this purpose. This work, now completed, was conducted under a PL 480 grant.

At the Ahmedabad Textile Industry's Research Association, Ahmedabad, India, an anhydride of 2-O-(2-hydroxyethyl)-D-glucose was isolated from the acid hydrolyzate of a commercial sample of O-(2-hydroxyethyl)starch. The compound, m.p. 212°-213°, $[\alpha]_D^{25}$ -56° (in water), is nonreducing, gives 2-O-2-hydroxyethyl)-D-glucose on acid hydrolysis, and consumes 1 mole of periodate per mole without liberation of formic acid. It is suggested that the compound is 1,2-O-ethylene-D-glucofuranose. Confirmation is being sought by NMR spectroscopy and by actual synthesis.

Attempts are being made to directly hydroxyethylate the 6-O-tritylate derivatives of amylose and starch to yield mainly the C-2 derivative after removal of the trityl group. Preparation of the C-6 substituted hydroxyethyl starch is also being investigated as part of the project. These studies are being conducted under a PL 480 grant.

4. Thermal reactions of starch. In studies under a grant to the University of Pittsburgh, Pittsburgh, Pennsylvania, pyrolysis of corn starch (200-400 g.) in a continuous screw-type conveyor reactor under vacuum gave lower yields of levoglucosan (28-33%) than pyrolyses in a small (5 g.) tubular batch reactor (39-41%). Poor maintenance of vacuum, clogging of the screw, and

decomposition of glucosans within the reactor were drawbacks to use of the screw conveyor. Pyrolyses conducted under inert atmospheres at atmospheric and half-atmospheric pressures in a kettle reactor gave low yields (11-22% vs. 39% under high vacuum). With superheated steam in the kettle reactor, yields were 9-13 percent at 1 atm., 18 percent at 0.75 atm., and 31-37 percent at 0.25 atm. A new reactor was designed and fabricated to study the effect of dielectric heating under nonarcing conditions.

Studies under a PL 480 grant to The University of Edinburgh, Edinburgh, Scotland, on thermal degradation of starch and model oligomers by differential thermal analysis has yielded the following information: (a) The production of CO₂ and CO from amylose and amylopectin degradation is a direct function of the H₂O evolved; (b) the production of CO₂, CO, and H₂O during pyrolysis at 225° to 300° C. under vacuum was essentially the same for G₅ and G₆ oligomers as for polysaccharides, amylose, amylopectin, and dextran; (c) the breakdown of oligomers G₁ to G₄ differs radically from that of the larger saccharides and hence only G₅ and larger oligomers may be considered as model compounds for thermal degradation studies of amylose; and (4) the syrup obtained from G₅ and smaller oligomers yields a complex mixture of products, and G₆, G₇, and polysaccharide syrups yield 1:6 anhydroglucose in which the pyranose to furanose ratios decrease with increase of α -1,6 linkages.

Additional studies on characterizing starch components show limited branching in amylose and the presence of linear material in "anomalous" amylopectin from amylo maize. Determination of starch concentration on a semimicro scale by an alkaline ferricyanide method is stated to be extremely satisfactory for use on a routine basis. Acid degradation studies on starch have begun. Confirmation of amylose in 0.33M KCl, 0.15M KOH, formamide, and dimethylsulfoxide is that of a random coil as indicated by hydrodynamic measurements.

5. Starch graft copolymers. Changes in graft copolymer structure (higher molecular weight polyacrylonitrile (PAN) grafts and less frequent grafting) with increased starch granule swelling are less pronounced with long reaction times and low reactant concentrations and are probably due to a reduced termination rate for growing PAN as granules become more swollen.

C. Microbiology and Fermentation

1. ARS culture collection. As of January 1, 1969, the ARS Culture Collection maintained a total of 37,130 strains of molds, yeasts, bacteria, and actinomycetes. The permanent collection contained 16,231 of these strains. The remainder are in temporary collections. During 1968, the Collection distributed 2,710 cultures of which 2,079 were sent to investigators in the United States and 631 were sent abroad. New major additions to the Collection during 1968 included 106 strains of gymnoascaceous molds, 404 strains of mucoraceous molds, and 122 strains of actinomycetes. The availability of

cultures from the Collection continues to generate publications of fundamental and practical import authored by members of the Collection staff as well as personnel in other laboratories of the Northern Division and elsewhere.

Variability in the segregation of several genetic markers was observed in a diploid yeast Hansenula holstii. The variability occurred in association with endospore formation in vegetatively growing cells and probably reflects the activity of a mechanism that modifies genetic linkages during mitotic growth. Hansenula wingei mutants with modified abilities to assimilate nitrate have been isolated. Several of these mutants map independently. Some control nitrate assimilation, others nitrite. Sex-specific diffusible agents are elaborated by complementary mating types in the yeast genera Hansenula and Kluyveromyces. The agents are specific and apparently influence the site of bud initiation in the target cell. Reciprocal stimulation between mating pairs causes new cells of the opposite sexes to grow toward one another. Chances for contact and conjugation between cells are augmented.

Under the grant to Baylor University, Houston, Texas, experiments were directed to cytological study of ascospore formation and spore germination in Hansenula holstii and Hansenula wingei. Cytological data indicate that both yeasts undergo regular meiosis prior to spore formation. Typically, two or three centrally located nuclei become incorporated into a comparable number of spores; the remaining one or two nuclei are excluded and pushed against the ascus wall. Thus, fewer than the normal complement of four spores invariably results. Sufficient spores have been obtained to study the changes which accompany spore germination in H. holstii. Germination is initiated by swelling of the spores, and buds are formed during the first 5 to 6 hours of incubation. The majority of spores germinate within 24 hours. The nucleus of a germinating spore divides by elongation and constriction. Mitochondria occur in the peripheral region of germinating spores and their buds. Mature buds are structurally like vegetative haploid cells.

Contract research by the American Type Culture Collection, Rockville, Maryland, revealed that of the 104 test strains of fungi previously reported to have survived preservation by the liquid nitrogen method for 30 months, 78 (all that could be tested in the time available) remained viable for 42 months. As a cryoprotective agent, 10 percent glycerol in water was adequate, except for eight strains. The latter were successfully preserved by use of 10 percent dimethylsulfoxide in water as a protective agent. This investigation, now completed, has demonstrated that economically important fungi that fail to withstand lyophilization can be preserved for at least 30 months, and probably more than 42 months, by the liquid nitrogen method.

In the past year, 17 streptomycete cultures were received from the Central Drug Research Institute, Lucknow, India, along with data from cultural studies in accordance with the provisions of a PL 480 grant. Each was tested by our routine shaken-flask screening procedure for antibiotic production. Ten demonstrated activity against a gram-positive bacterium

(Bacillus subtilis), 4 against a gram-negative plant pathogenic bacterium (Agrobacterium tumefaciens), 6 against a mold (Mucor ramannianus), and 3 against a yeast (Saccharomyces pastorianus). All have been placed in our antibiotics culture collection as potentially useful organisms for future screening projects.

Ninety-eight streptomycete cultures, along with data from cultural studies, were submitted by the National Institute of Agronomic Research, Madrid, Spain. Each was tested by our routine shaken-flask screening procedure for antibiotic production. Forty-eight demonstrated activity against a gram-positive bacterium (Bacillus subtilis), 6 against a gram-negative plant pathogenic bacterium (Agrobacterium tumefaciens), 48 against a mold (Mucor ramannianus), and 19 against a yeast (Saccharomyces pastorianus). All have been placed in our antibiotics culture collection as potentially useful organisms for future screening projects.

2. Studies on enzymes. Quantitation of the autohydrolyzate components of Y-1842 phosphomannan (PM) has been achieved by alcohol precipitation of the α -mannan fragment and Sephadex fractionation of the oligomers. Characterization of the 2-O-(α -D-mannosyl)-D-glucose end group of Y-2023 PM has been completed. A survey of end groups has been completed for PM's wherein the phosphodiester bridges occur peripherally. Smith degradation studies on Y-2579 PM indicate that its high proportion of α -(1 \rightarrow 2)-linked mannose residues account for the strong interactions with concanavalin A and Lens culinaris hemagglutinin. Neutral mannans were produced when Y-1842 and Y-2448 were grown on media lacking added orthophosphate. A rapid Sephadex fractionation method has been developed for separation of high and low molecular weight autohydrolysis products from Y-2448 PM. Lipomyces starkeyi polysaccharides crossreact with antipneumococcal Type XVIII sera. The acetylated polymer contains periodate-resistant galactose residues. Smith degradation of phosphogalactan yielded a periodate-resistant galactan unit.

Separation and purification of Streptomyces N-acetylmuramyl-L-alanine amidase, aminopeptidase, and four bacteriolytic endopeptidases were improved. It was concluded from degradation of Staphylococcus aureus cell walls with various enzymes that 4-O- β -(N-acetyl-D-glucosaminyl) substituents on D-ribitol units of the teichoic acid polymer are the sites of phage adsorption and that the supporting peptidoglycan network serves to hold these sites in a definite configuration. In work on linkages between peptidoglycan and other wall polysaccharides, two different polysaccharide-peptidoglycan complexes were isolated from Streptococcus pyogenes walls. Both complexes contained phosphodiester bridges. Wall peptidoglycans of Micrococcus flavus, M. citreus, and Sarcina lutea were found to resemble that of M. lysodeikticus and to differ from those of other micrococccaceae. Butyrivibrio rettgeri wall peptidoglycan is composed of N α -(L-seryl- γ -D-glutamyl)-L-ornithyl-D-alanine subunits. D-lysine and D-ornithine bridges occur in the ratio of 2 to 1. These extend via a glutamic α -carboxy- ω -amino link on one subunit to the

carboxyl of C-terminal D-alanine on another subunit via the α -amino group of the diamino acid. This research is conducted under a PL 480 grant to the University of Leige, Leige, Belgium.

3. Fermentation with spores. Sucrose was transformed to invert sugar in shaken flasks and in columns by spores of Aspergillus oryzae. In shaken flasks, the inversion rate was linear. Spore columns could be increased in size with no loss of inversion capacity. Spore columns retained their sucrose inversion ability after 1 year dry storage at room temperature. Attempts to hydroxylate glycyrrhetic acid by various fungal spores was unsuccessful but spore activity was observed on isoborneol. Glucose has been transformed to mannitol by spores of A. candidus. Quantitative methods to determine mannitol in the presence of glucose are being developed. Decarboxylation of diaminopimelic acid to lysine by bacterial spores was unsuccessful.

4. Biological insecticides for Japanese beetle. Techniques have been established which cause significant sporulation in vitro by several strains of Bacillus popilliae. All strains were derived by selection from standard strain NRRL B-2309 or from other sporogenic strains. Sporulation in colonies on agar average 15 to 30 percent in 11-day incubation periods. These spores cause milky disease by injection, but premature germination of these spores may interfere with infectivity when fed in soil. More than 10^7 spores/ml. are produced in 12 to 21 days by one strain during growth in a liquid medium containing particulate carbon. Optimum sporulation by both techniques requires specific yeast extracts and spore inocula. These procedures provide means for studying sporogenesis hitherto impossible and for evaluating factors in spores which influence infectivity. Metabolic studies show that milky disease organisms growing in artificial culture lack the oxidative enzymes characteristic of sporulation in other bacteria. Hemolymph may provide an environment for induction of these enzymes or alternative energy sources such as fatty acids. Enumeration and microscopic study of sporulation in larvae indicate that the rate of sporulation in vitro approximates that in larvae where sporulation occurs over an extended period and large numbers of spores accumulate.

Studies under a contract at Michigan State University, East Lansing, Michigan, indicate that the capacity to sporulate in Bacillus popilliae involves ability to obtain energy by the oxidation of substrates such as acetate and glutamate in a manner analogous to the sporulation processes in other sporeformers. The required enzymes are at least partially present in sporogenic strains undergoing sporulation but are not present during vegetative growth nor under conditions in which spores are not formed by sporogenic strains. The enzymes are not present in asporogenic strains. Other enzymes such as protease associated with sporogenesis in other organisms are not detected in B. popilliae. These results indicate that cultural conditions may have to be devised which permit induction of the enzymes and that methods can be developed for selecting sporogenic cultures based on the occurrence of these enzymes in cells of certain ages.

5. Fermentation acids. In studies completed during the year at the University of Lodz, Lodz, Poland, attempts to find organisms that produce significant amounts of itatartaric acid from glucose were only partially successful. Mutant strains of Aspergillus terreus were isolated which could produce up to 2 g./100 ml. of fermented liquor. However, the fermentation time was quite long, taking 14 to 21 days in surface cultures. These conditions would not be practical for commercial production. The nutritional requirements and physical parameters necessary for maximum itatartaric acid production were thoroughly studied. It is quite apparent that if itatartaric acid is to be made by fermentation, a detailed study of the metabolic pathway leading to its formation must be conducted. This research was conducted under a PL 480 grant.

Work, under a PL 480 grant at the University of Tokyo, Tokyo, Japan, was concentrated on finding mutants of tartaric acid-producing organisms which would have greater capability of producing this acid. One such mutant was obtained by repeated treatment of the organism with N-methyl-N'-nitro-N-nitroso-guanidine (NTG). This new strain was capable of producing 11 grams of tartaric acid from 50 grams of glucose in 1 liter of broth. The direct precursor of tartaric acid appears to be 5-ketogluconic acid. 2-Ketogluconic acid is also found but this does not convert to tartaric acid. The mutant strains produce less 2-ketogluconic acid than the parent strains, which accounts for the higher yield of tartaric acid. A polarographic method for determination of tartaric acid in cultured broth was developed and is the most suitable method for routine analyses.

6. Preparation of microbial polysaccharides. Under a grant to Cornell University, Ithaca, New York, bacterial and yeast cells coated with positively charged colloidal aluminum oxide were found to be exceptionally resistant to loss of viability and autolysis. This is an unexpected observation since most cationic agents readily rupture microbial cells, causing loss of the cell contents. The coated cells maintain their physiological capabilities such as uptake of sugar and formation of adaptive enzymes. Coated yeast cells were found to combine with uncoated cells in a 1:1 ratio causing a high degree of flocculation. This is significant because it is a method to harvest yeast grown in dilute waste streams without resort to costly techniques such as evaporation or centrifuging. Coated cells could be attached to fibrous glass or cellulose surfaces. Such attached cells of Lactobacillus delbrueckii were shown to be active in converting glucose to lactic acid.

D. Technology--Process and Product Development

1. Protein-dialdehyde starch (DAS) glue for southern pine plywood. Laboratory and pilot-plant studies have shown that it is necessary to maintain veneer moisture at 5-7 percent and veneer specific gravity at about 0.5 to produce interior-type southern pine plywood, bonded with dialdehyde starch-protein glue, that will meet the performance requirements of the interior plywood test, Product Standard PS 1-66, of the American Plywood Association.

2. Graft copolymers. Under contract research at General Mills, Minneapolis, Minnesota, starch was grafted variously with acrylonitrile (AN), acrylamide (AA), acrylic acid (AAc), and quaternary olefin (Q) to provide an inventory of 46 products having sufficient diversity to permit a thorough and conclusive evaluation of their utility in industrial applications. Laboratory evaluations revealed that several of the starch graft polymers have considerable potential in a number of end-use applications. Starch-AA copolymers may be useful as short-term acid thickeners such as those used in industrial cleaning processes. Starch-AA and starch-AA-Q polymers give excellent performance as retention aids for clay-filled papers. A starch-Q copolymer and a starch-AA-Q terpolymer are superior to the best commercial flocculants presently available for flocculating silica. Starch-AA-Q terpolymer is highly effective as a silica depressant in fatty acid flotation beneficiation of Florida phosphate ore. Starch-AA-AAc terpolymers (from hydrolyzed starch-AN) thicken and emulsify aqueous organic mixtures such as those used in various cosmetics, pharmaceuticals, and chemical specialties and should be very competitive with synthetic resins presently used in these applications. Starch-AA-AAc terpolymers are effective as stabilizers and thickeners for latex paints at one-sixth the level of addition of the polymer presently used for such purposes.

3. Starch for rubber reinforcement. At the University of Akron, Akron, Ohio, under contract, two masterbatch types were selected (based on processing and vulcanizate properties) for detailed study and process design. The selected masterbatch types were (a) 50 parts starch xanthide (as starch) per 100 parts (phr) styrene-butadiene elastomer and (b) 50 phr resorcinol-formaldehyde treated starch xanthide in acrylonitrile-butadiene elastomer. They showed excellent potential for use in general purpose and automotive goods, giving tensile strengths greater than 2,000 p.s.i. and 2,500 p.s.i., respectively. The starch nitrile masterbatch gave superior oil-resistant rubbers. Process design for commercial-scale production gave cost estimates showing that these masterbatches would be competitive with traditional products.

The effects of time, temperature, radiation dose rate and total dose, swelling agents for starch, and monomer concentration on the grafting of butadiene (BD) to granular starch in aqueous dispersions were investigated under contract at Stanford Research Institute, Menlo Park, California. Under no conditions were products with more than 3.2 percent grafted BD obtained. Similar results were obtained when starch in a fluidized bed was reacted with BD by direct irradiation with an electron beam. However, when active comonomers such as acrylonitrile (AN) and methyl acrylate (MAc) were added to the BD, products containing up to 29 percent grafted BD and 15 percent grafted comonomer were obtained. No advantages, as far as degree of grafting is concerned, were noted when gelatinized starch was substituted for granular starch. However, differences in product properties may be found which would give advantages in certain applications. Preliminary evaluation of selected products as internal sizes for paper showed that some of the starch-BD-AN terpolymers improve wet bursting strength of paper.

4. Microbial polysaccharides. Single-stage continuous fermentations to produce xanthan biopolymer have been successfully run in the pilot plant at dilution rate of about 0.025 per hour and in the laboratory at dilution rates as high as 0.16 per hour. Xanthan production rate was a function of dilution rate. The xanthan production rate increased as dilution rate increased. The daily yield based on glucose consumed was 70 percent at low dilution rate and 84 percent at high dilution rate. Medium modifications have been made which gave a more rapid transposition to steady state at an increased viscosity. Contamination of the fermenting broth after about six turnovers of the fermentor contents has been a problem. Penicillin G at 20 units per gram did not control contamination. Studies on process improvement of the batch fermentation have resulted in a twofold increase of polymer concentration in the fermentor.

5. Starch derivatives. Studies on new methods for evaluating cationic starches have developed preliminary information on methodology for screen testing the adsorption of starches on woody fibers. These test methods showed that ordinary starches were not adsorbed on wood fibers, while laboratory-prepared and commercial cationic starches were adsorbed. Cationic amylose was adsorbed more strongly, but not as fully as cationic amylopectin. Attention should thus be given to the kind of starch used for wet-end addition to paper. Aminoethyl cationic starches were prepared by a vapor-phase process which yields a product with good physical properties.

Pilot-scale evaluations of starch xanthate preparation and its application in papermaking are in final stages. Maximum properties obtainable with oxidatively crosslinked starch xanthide are below expectations predicted from previous results on the 10-inch laboratory paper machine. Drying conditions were critical to development of maximum properties, but dryer conditions on the laboratory equipment are difficult to duplicate in commercial paper machines. Starch xanthate-polyethylenimine (X-PEI), a readily prepared derivative, is relatively easy to use and does not require oxidative coupling. X-PEI performed as expected in a commercial mill trial. Cationic aminoethyl flours (flour-ethylenimine reaction products) were evaluated in extensive pilot paper machine trials and are as effective as commercial cationic starches. The derivatized protein is believed to be an effective component. Preliminary handsheet tests have confirmed the high effectiveness of several starch graft polymers as filler retention aids.

In contract research at Battelle Memorial Institute, Columbus, Ohio, the physical test data obtained with interior and exterior paints containing starch derivatives, and with panels painted with these paints and exposed for 18 months at various conditions of temperature and humidity, show some deficiencies of the paints. However, it is expected that these can be compensated for by employing conventional paint-formulation principles. It has been concluded by the contractor that starch derivatives have some promise for use as viscosity-control agents in emulsion paints and can result in a decrease in raw material cost of as much as 2 cents per gallon of paint.

From a total of 390 alkyd resins prepared from starch-derived glycosides, under contract by Archer Daniels Midland, Minneapolis, Minnesota, 53 received extensive clear film evaluations. Based on these evaluations, 26 of the best resins were further evaluated in pigmented paint studies. These tests revealed that alkyd resins suitable for industrial coatings can be derived from glycol glycosides. The resins performed well in all respects when compared to standard controls. They dry and cure faster under bake cycles than do the controls; however, their yellowing and gloss properties were slightly inferior.

Of all the alkyds prepared, urethane oils based on glycosides appeared best. Several of these were judged commercially acceptable. Film properties, particularly dry time and hardness, were superior to those obtained from a commercial control. Their initial gloss was generally inferior to controls; however, after 65 hours exposure, the difference in gloss retention became negligible.

Based on the results of pigmentation studies, five resins were selected for 30-gallon pilot-plant synthesis. Technical difficulties were encountered in scaling up the synthesis from laboratory to pilot plant. All resins prepared were too dark for general coating applications. The contract does not provide for more extensive pilot studies. Undoubtedly, this can be resolved by process modifications which more closely parallel laboratory procedures.

RPA 702 - PROTECT FOOD SUPPLIES FROM HARMFUL MICROORGANISMS
AND NATURALLY OCCURRING TOXINS

A. Microbiology and Toxicology

1. Aflatoxin investigations. The structure of three new compounds related to aflatoxin were found. One of these is parasiticol, a compound with a difurocoumarin ring with a hydroxyethyl side chain. It has the toxicity of aflatoxin B₁. Aflatoxin B_{2a} is a compound produced through mild acid treatment of aflatoxin B₁ and only 1/200 as toxic as B₁. The third compound is a hydroxylated aflatoxin made by many fungi from aflatoxin B₁ and 20 times less toxic. Survey of corn from the 1967 crop year shows about the same incidence of aflatoxin as reported for years 1964 and 1965. Ochratoxin was found in one corn sample in commercial channels and in another sample going into the export market. Tracer studies with resting cell suspensions of Aspergillus parasiticus show that the mold can shift from the biosynthesis of aflatoxin to lipids when high aeration rates are used or when high exogenous levels of acetate are present. Metabolic studies on the binding of aflatoxin B₁ to cells of Bacillus indicated that aflatoxin perturbed nucleic acid synthesis without covalent attachment to cellular components or metabolic conversion. Certain species of Penicillium present in cereal products form a tremorgenic toxin which has been isolated as a crystalline product. The first culture studied came from moldy feed implicated in the death of dairy cows. Sterigmatocystin has been isolated and found to be only 1/25 as toxic as B₁ in inducing bile duct neoplasia.

In contract research at the University of South Dakota, Brookings, South Dakota, the survey for toxicity of most of the common species of Aspergillus is nearing completion. As many as 10 strains of each species were grown on wheat and soybeans and fed as 50 percent of the diet to mice and chicks. Toxicity has been encountered in the last year in the A. wentii, A. tamarii, A. flavipes, A. niveus, and A. nidulans groups with the most toxic material in the species A. flavipes and A. niveus. In some instances, good correlation exists between the toxic response of the two test animals and in other toxic feeds only one animal responds. In the A. glaucus group containing many of the common storage molds, little or no toxicity occurred and, in fact, many strains promoted growth of chicks.

2. Reduction of viable organisms in milled cereal products. Work was concluded on a study of the chlorine tolerance of microorganisms from wheat and flour and gave information on conditions needed for reduction of the microbial population. A study was also completed on the identification and distribution of yeasts from domestic wheat and flour. Work was completed on the microbiology of refrigerated dough products and included a comprehensive study of the species of Bacillus present. Exploratory work was begun on the spoilage of mayonnaise and salad dressings because of the large amount of corn starch used in the formation of these products. Spoilage of these products appears to be caused by a single species of yeast and a few species of bacteria. A study of the microbiology of stored corn was begun, and included various high-temperature treatments of freshly harvested corn and storage of these samples under different low-temperature conditions as might be found under commercial conditions. In addition, a microbiological examination of corn meals and corn mixes was undertaken on samples collected in local markets.

3. Microbiological processes and products. Work in four major areas has yielded the following results: (a) The intracellular concentration of alanine in Rhodospirillum rubrum and its bacilliform mutants was investigated to ascertain how alanine regulates the morphology of this bacterium. The relative contribution of alanine-producing transaminases to alanine levels in the cell was evaluated.

(b) The controlled biosynthesis of fatty acids was studied through the use of Agrobacterium tumefaciens mutants that require methionine for methylation and pantothenic acid for hydrocarbon chain elongation. Results indicate that monounsaturated acid production is maximal when exogenous methionine is the limiting factor of growth, and that the ratio of C₁₈ to C₁₆ acids is dependent on pantothenate concentration.

(c) The association reaction of the sex-specific agglutinative particles from opposite mating types of the yeast Hansenula wingei was found to have a very strong binding energy of 14 kilocalories per mole.

(d) Three new microbial products have been isolated and their structures investigated: (1) 2-hydroxyhexadecanoic acid from a new yeast, (2) a

crystalline phenolic acid from the mold Aspergillus unguis, (3) a substance from Penicillium egyptiacum with an unusual combination of nitrogens.

Candida lipolytica has been isolated as a sexually active strain which will be described and given a name according to the perfect genus in which it belongs. Work has been completed on a study of the vitamin requirements and abilities of the perfect strain and some imperfect (nonascosporogenous) strains to assimilate various carbohydrate and hydrocarbon compounds. Mating types have been isolated, proving the heterothallic nature of its sexual reactions. This species assimilates hydrocarbons and produces extracellular proteinase and lipase.

4. Bacillus species identification. Under a grant to Rutgers University, New Brunswick, New Jersey, analysis of data resulting from the characterization of 581 strains of aerobic sporeforming bacteria has allowed the assignment of 504 of them (87%) to 15 species. Descriptions of these have been prepared. The remaining 77 strains fall into several groups, none of them large enough to provide data sufficient to justify species recognition. They are relegated to a category designated "Unidentified strains that may or may not belong to a definite species." Data are in process of being assembled as a manuscript.

RPA 901 - ALLEVIATE SOIL, WATER AND AIR POLLUTION

A. Chemical and Physical Investigations to Improve Products

1. Biodegradable surfactants and detergent builders. In the quest for replacement of sodium tripolyphosphate (STPP) detergent builder by carbohydrate derivatives which presumably will be biodegradable, evaluations were made of the sodium salts of the following acids in a standard linear alkylbenzenesulfonate detergent (LAS) formulation: citric, glucoheptonic, glucaric, gluconic, and polyitaconic acid. Although all of these acids except citric have good sequestering action toward calcium ions in strongly alkaline solution, only the sodium salts of citric, polyitaconic, and a commercial glucoheptonic acid showed appreciable cleaning of standard soiled cotton fabric in water of 150 and 300 p.p.m. of hardness when they replaced 50 percent of STPP in the detergent formulation. Of the starch derivatives investigated, a commercial carboxymethyl starch and dicarboxyl starch prepared by chlorous acid oxidation of dialdehyde starch had significant builder action.

At Ashland Chemical Company, Minneapolis, Minnesota, under contract, procedures were outlined for modifying glycol and glycerol glycosides derived from starch, with lyophilic and hydrophilic groups to obtain biodegradable surfactants for use in household and industrial detergents. Because light color is important, reaction conditions were designed to yield surfactants with a Gardner color of 3 or less. A modified procedure for the glycerolysis of starch yielded glycerol glycosides with a Gardner color of 1. An acceptable procedure for preparing glycol glycosides was established in a previous

study. Nine surfactants from a proposed list of 47 have been prepared and evaluated for effect on air and oil interface surface tensions. To date, the most promising compound prepared was obtained by reacting glycol glycosides with 10 moles of ethylene oxide followed by 1 mole of methyl stearate. This compound satisfactorily reduced surface tension and performed well as a detergent in regard to soil removal in Terg-o-tometer studies.

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RPA 406 - NEW AND IMPROVED FOOD PRODUCTS FROM FIELD CROPS

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RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL
PRODUCTS FROM FIELD CROPS

Industrial Products

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RPA 702 - PROTECT FOOD SUPPLIES FROM HARMFUL MICROORGANISMS AND NATURALLY OCCURRING TOXINS

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WHEAT UTILIZATION (NORTHERN REGION)

USDA and Cooperative Programs

Problem Area		Scientist Man-Years FY 1969 (Estimated)		
No.	Title and Activity	Intra- mural	Extra- mural	Total
406	New and Improved Food Products from Field Crops			
	Chemical Composition and Physical Properties	7.5	0.2	7.7
	Microbiology and Toxicology	2.5	1.3	3.8
	Technology--Process and Product Development	1.7		1.7
	Subtotal	11.7	1.5	13.2
407	New and Improved Feed, Textile and Industrial Products from Field Crops			
	<u>Industrial Products</u>			
	Chemical Composition, Physical Properties and Structure		1.5	1.5
	Chemical and Physical Investigations to Improve Products	8.0	2.8	10.8
	Microbiology and Fermentation	10.5	0.6	11.1
	Technology--Process and Product Development	4.9	2.8	7.7
	Subtotal	23.4	7.7	31.1
702	Protect Food Supplies from Harmful Microorganisms and Naturally Occurring Toxins			
	Microbiology and Toxicology	4.8	0.3	5.1
	Subtotal	4.8	0.3	5.1
901	Alleviate Soil, Water, and Air Pollution			
	Chemical and Physical Investigations to Improve Products	1.4	0.3	1.7
	Subtotal	1.4	0.3	1.7
	Total	41.3	9.8	51.1

Domestic program supplemented by PL 480 funds in 1 country totaling
51,887 U.S. dollars equivalent (Poland).

Problems and Objectives

The dominant factor in the wheat economy of the United States continues to be a production capacity that can outpace U.S. and foreign demand. Research on wheat seeks to solve the problems hindering the development of markets for the full productive capacity of U.S. agriculture. The Northern Division research seeks to find new industrial markets for wheat, particularly in the Pacific Northwest where corn is not grown, and to improve yield and quality of wheat flour for use in foods.

Major objectives of current research are to develop and evaluate alternate ways to:

1. Convert wheat flour and starch to chemical products whose use will be economically advantageous to selected segments of the paper industry.
2. Improve yield and quality of wheat milling fractions.

Progress - USDA and Cooperative Programs

RPA 406 - NEW AND IMPROVED FOOD PRODUCTS FROM FIELD CROPS

A. Chemical Composition and Physical Properties

1. Microscopic and ultrastructure of wheat. Differences in wheat protein quality may depend more on structural than on chemical variation. This idea is being investigated by examining the macromolecular organization of wheat protein with the electron microscope. Observations have been made on protein films dispersed on a water surface as a means of examining the protein at high resolution. Small protein units in the range 65-250 Å have been observed on such water dispersions and provide a convenient approach for studying the ultrastructure of various wheat proteins. Molecular weights of these protein units are in the range reported in the literature. Protein surface dispersions of hard, soft, and durum wheat all showed similar protein particles when stained with KMnO_4 . Chemical fixation of thin, dry, wheat endosperm sections on aqueous solutions showed that, in hard wheats, the protein expanded to form a network with compact marginal areas which outlined the starch granule. A soft wheat protein showed no network on compact areas.

2. Characterization of wheat gluten proteins. Fragments from enzymic digests of glutenin and gliadin from a HRW wheat have very similar or identical structures. Each gluten fraction also contains some unique structures that probably are responsible for its characteristic physical properties. In glutenin, peptides that occur between pepsin-susceptible bonds are larger than those in gliadin, and their amino acid compositions are different from those of corresponding gliadin peptides.

Individual gliadin proteins also contain many similar or identical peptides even though the intact proteins have different properties and amino acid compositions. Each, however, has some unique structure. Slight differences are apparent between good and poor quality HRW wheats when fragments from proteins that have the same chromatographic and electrophoretic properties are compared. Such differences may account for gluten quality variations between varieties. Changes noted upon reduction and S-aminoethylation support the hypothesis that gliadin molecules are compactly folded and suggest that most cysteine residues in gluten occur in structures which vary from one protein to another.

For intact glutenin proteins within a class of wheat, gluten quality correlates better with response to salt than with gliadin/glutenin ratio, viscosity, or electrophoretic distribution. This correlation may prove useful in relating gluten properties to specific proteins.

Circular dichroism of gliadin in several solvents indicates that alpha helix and unordered structure are present in gliadin but beta structure is not. These findings are consistent with optical rotatory dispersion and infrared spectra data.

3. Other relevant research. Research on wheat starch is integrated with that on corn starch. Results are reported under "Corn Utilization," RPA 406, Section A.

B. Microbiology and Toxicology

Research in this category is integrated with similar research under "Corn Utilization," RPA 406, Section C.

C. Technology--Process and Product Development

1. Milling and fractionation. Peeled wheats of three different classes, prepared by mild-temperature caustic peeling, differed in milling and fractionating characteristics from their unpeeled controls. The differences varied in magnitude between classes, with HRW changing the most. All peeled samples gave greater yields of shorts and also higher ash and lower fat in the flours. SRW and SWW varieties had protein shift values similar to their respective control lots. When pin-milled flour from peeled HRW wheat was fractionated, results resembled those for a wheat of higher hardness; namely, lower yields for the finer fractions, corresponding marked increase in coarse residue, and lower protein shift. Tentative specifications were developed for an 85-percent wheat-flake/15-percent soy-flake blend. Stability of vitamin A and ascorbic acid added to wheat flour was satisfactory when the flour was stored at not over 14 percent moisture and 40° C.

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL
PRODUCTS FROM FIELD CROPS

Feed

A. Microbiology and Toxicology

Research in this category is integrated with similar investigations on Corn. Results are reported under "Corn Utilization," RPA 407, Feed, Subheading A.

Industrial Products

A. Chemical Composition, Physical Properties and Structure

1. Chemistry of glycoprotein linkages. Experiments conducted under grant at Iowa State University, Ames, Iowa, show fluidization of flours and starches to be different from fluidization of free-flowing materials, such as polyethylene powders, in several important respects. Agglomerative forces are evident, requiring higher energy inputs to achieve the benefits of fluidized bed operation. Studies of heat exchange in a model fluidized bed are at the state of development so that results on flours and starches should be available soon. The thermal conductivity of starch pastes has been extensively investigated and a correlation accounting for the effect of starch concentration has been developed. Increased knowledge of the physical properties of starches and flours facilitates more economical design and operation of processes using them.

At the University of Tennessee, Knoxville, Tennessee, under grant, improved preparation of 2,3,4,6-tetra-O-benzyl- α -D-glucopyranosyl chloride has facilitated syntheses of O-glycosides of β -hydroxy- α -amino acids. Reaction of 2,3,4,6-tetra-O-benzyl- α -D-glucopyranose with zinc chloride-thionyl chloride complex in benzene results in quantitative conversion of the anomeric hydroxyl group to chloride within an hour. The α -glucosyl chloride was used to form α -O-glucosides of L-serine and L-threonine. Debenzylation of the base-sensitive glycosides by hydrogenation surmounted the difficulties previously encountered upon deacetylation of the corresponding O-acetyl derivatives. By a second route, the O-mannosides of serine and threonine were formed in 20-30 percent yields by addition of the suitably blocked amino acid to 3,4,6-tri-O-acetyl-D-glucal, followed by hydroxylation of the (displaced) 2,3-double bond. By a third route, D-xylosides and D-glucosides of 2-nitroethanol were synthesized. These glycosides were carboxylated and the nitro group reduced to produce crude O-glycosides of serine. Purer products are being sought by this route of synthesis. Investigations were begun on fragmentation of the glycosides by pyrolytic gas chromatography and by mass spectrometry.

2. Other relevant research. Research on wheat starch is integrated with that on corn starch. Results are reported under "Corn Utilization," RPA 407, Industrial Products, Section A.

B. Chemical and Physical Investigations to Improve Products

1. Chemical modification of wheat gluten. A photochemical study with wheat gluten proteins in 0.01N HCl and in dimethyl sulfoxide (DMSO) showed that (a) a linear relationship exists between the increased absorption at 250 m μ and the sulfur content of the photoproducts; (b) sulfur-containing and aromatic amino acids are modified to a greater extent in the DMSO than in the aqueous medium; and (c) styrene is grafted to proteins in DMSO. The graft photopolymers are soluble in organic solvents.

An investigation of the mechanism of anionic graft polymerization of vinyl monomers to wheat gluten proteins in DMSO indicates that electronic effects predominate over steric factors in stabilizing the carbanions formed during the propagation step.

4-Vinylpyridine was found to be a useful reagent for the selective modification and quantitative determination of sulfhydryl groups in reduced proteins and seed meals. S- β -(4-pyridylethyl)-L-cysteine was found to be valuable as an internal standard for amino acid analysis by ion-exchange chromatography.

The carboxyl group of mono-, di-, and trihalogenated acetic acids, such as trifluoroacetic acid, adds to the double bond of alkyl vinyl ketones forming esters of 2-butanone.

Under grant research at the University of Chicago, Chicago, Illinois, a number of reactive cyclic esters of sulfur- and phosphorus-containing acids have been synthesized. These include 2-hydroxy- α -toluenesulfonic acid sultone, the corresponding 5-nitro sultone, 4-nitrocatechol cyclic sulfate, and catechol cyclic phosphate. These esters react rapidly with functional groups present in proteins. The serine of α -chymotrypsin reacts with 4-nitro-catechol cyclic sulfate to give a sulfonated protein. A site-specific modification of the active site serine was achieved in chymotrypsin with a cyclic sulfate. 4-Nitrocatechol cyclic sulfate appears to be a promising new reagent for the chemical transformation of serine residues in proteins. 2-Hydroxy-5-nitro- α -toluenesulfonic acid sultone reacts with the sulfhydryl protein, papain, by at least two pathways. One of these pathways results in the inactivation of the enzymatic activity of the protein.

2. Other relevant research. Research on wheat starch is integrated with that on corn starch. Results are reported under "Corn Utilization," RPA 407, Industrial Products, Section B.

C. Microbiology and Fermentation

D. Technology--Process and Product Development

Research in these categories is integrated with similar investigations on corn. Results are reported under "Corn Utilization," RPA 407, Industrial Products, Sections C and D.

RPA 702 - PROTECT FOOD SUPPLIES FROM HARMFUL MICROORGANISMS
AND NATURALLY OCCURRING TOXINS

Research in this category on wheat is integrated with similar studies on corn. Results are reported under "Corn Utilization," RPA 702.

RPA 901 - ALLEVIATE SOIL, WATER AND AIR POLLUTION

Research in this category is integrated with similar investigations on corn. Results are reported under "Corn Utilization," RPA 901.

Publications - USDA and Cooperative Programs

RPA 406 - NEW AND IMPROVED FOOD PRODUCTS FROM FIELD CROPS

Chemical Composition and Physical Properties

Mattern, P. J., Salem, A., Johnson, V. A., and Schmidt, J. W. (University of Nebraska, Lincoln, Nebraska). 1968. Amino acid composition of selected high-protein wheats. *Cereal Chem.* 45(5), pp. 437-444.

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Rothfus, J. A., and Crow, M. J. A. 1968. Aminoethylation and fractionation of glutenin. *Biochim. Biophys. Acta* 160(3), pp. 404-412.

Microbiology and Toxicology

Wang, H. L., Ruttle, D. I., and Hesseltine, C. W. 1968. Protein quality of wheat and soybeans after Rhizopus oligosporus fermentation. *J. Nutr.* 96(1), pp. 109-114.

Wang, H. L., Ruttle, D. I., and Hesseltine, C. W. 1969. Milk-clotting activity of proteinases produced by Rhizopus. *Can. J. Microbiol.* 15(1), pp. 99-104.

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL
PRODUCTS FROM FIELD CROPS

Industrial Products

Chemical Composition, Physical Properties and Structure

Crow, M. J. A., and Rothfus, J. A. 1968. Chromatography of proteins from wheat gluten on polyacrylamide gel. *Cereal Chem.* 45(5), pp. 413-420.

Chemical and Physical Investigations to Improve Products

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GRAIN SORGHUM UTILIZATION

USDA and Cooperative Programs

Problem Area		Scientist Man-Years FY 1969 (Estimated)		
No.	Title and Activity	Intra- mural	Extra- mural	Total
406	New and Improved Food Products from Field Crops			
	Chemical Composition and Physical Properties	2.8		2.8
	Microbiology and Toxicology	0.6	0.3	0.9
	Technology--Process and Product Development	5.1	1.2	6.3
	Subtotal	8.5	1.5	10.0
407	New and Improved Feed, Textile and Industrial Products from Field Crops			
	<u>Industrial Products</u>			
	Chemical and Physical Investigations to Improve Products	2.0	0.6	2.6
	Microbiology and Fermentation	2.6	0.1	2.7
	Technology--Process and Product Development	1.2	0.7	1.9
	Subtotal	5.8	1.4	7.2
702	Protect Food Supplies from Harmful Microorganisms and Naturally Occurring Toxins			
	Microbiology and Toxicology	1.2		1.2
	Subtotal	1.2		1.2
901	Alleviate Soil, Water and Air Pollution Chemical and Physical Investigations to Improve Products	0.4		0.4
	Subtotal	0.4		0.4
	Total	15.9	2.9	18.8

Problems and Objectives

The growing importance of grain sorghum as a cash crop and the increasing magnitude of production challenge technology to establish a pattern of utilization that will maintain or increase the economic value of the crop. Because the bulk of the U.S. crop is grown in a relatively limited region west of the Mississippi River, there are geographic areas where freight transportation advantages should permit increased food and industrial usage of grain sorghum without significant displacement of other cereal grains. Food usage, now taking only about 1 percent of production, could be increased by improving quality, acceptability, and suitability of grain sorghum products for food. Limited industrial outlets for grain sorghum already exist. Processing techniques specifically adapted to grain sorghum should result in improved products that can maintain present industrial outlets against competition from synthetics as well as lead to increased industrial utilization.

Major objectives of current research are to develop and evaluate alternate ways to:

1. Get needed data on composition and processing treatments for use in making new food products from grain sorghum.
2. Convert grain sorghum to new or improved industrial products via technology suited to the specific characteristics of this grain.

Progress - USDA and Cooperative Programs

RPA 406 - NEW AND IMPROVED FOOD PRODUCTS FROM FIELD CROPS

A. Chemical Composition and Physical Properties

1. Chemical and physical studies on sorghum proteins. Proximate composition and proteins of three grain sorghum hybrids and their dry-mill fractions were studied. Sorghum was milled in a Buhler mill to obtain bran and shorts, together with first and second flour fractions. Each fraction was extracted successively with water, 1 percent sodium chloride, and 60 percent alcohol (t-butyl alcohol at room temperature or ethanol at 60° C.). The three sorghums had similar chemical compositions and milling properties. Also similar were solubility, electrophoretic patterns, and amino acid content of the protein.

Protein composition of proso millet was obtained from dehulled white proso millet flour. The flour was extracted successively with water, 1 percent sodium chloride, and alcohol. Both 60 percent t-butyl alcohol and 60 percent ethanol were used. Electrophoretic patterns and amino acid composition of each extract were obtained. Protein bodies were identified by both light and

electron microscopy. They are composed mainly of prolamines which dissolve in hot t-butyl alcohol.

It was determined in contract research at Kansas State University, Manhattan, Kansas, that fertilization, irrigation, or hybridization had no significant effect on protein content, amino acid composition, or weight gains and feed conversions with chicks. Low test weight samples when compared to normal samples contained more lysine, aspartic acid, and glycine, and less proline and leucine. Estimated energy of the low test weight grain was 1,000 calories per pound and 1,100 calories per pound for the normal grain. Differences in protein efficiency ratios (PER) of isocaloric diets of the low and normal test weight grain were not significant at the 1-percent level. Dry-milling studies have been conducted using a modified Mag Multomat Experimental Flour Mill. Protein content ranged from 3.3 percent in the first break flour to 8.8 percent in the 5 midds flour. The overs of the 13XX sieve is a hard flinty product which probably contains a large portion of the aleurone layer. This fraction has a high protein content (12.8%) and a low fat content (0.14%). On an isonitrogenous basis, protein quality of floury endosperm was superior to that of horny endosperm. Significant differences were not present when floury and horny endosperm fractions were supplemented with sufficient lysine and methionine to meet 100 percent of NRC requirements.

Under a PL 480 grant at the Indian Institute of Science, Bangalore, India, kafirin, the prolamine or alcohol-soluble protein of grain sorghum, has been characterized by studying reversible reduction and reoxidation of its disulfide bonds. Results indicate that these bonds are chiefly intramolecular, similar to the disulfide bonds of wheat gluten. This research was completed during the year.

2. Other relevant research. Research on sorghum starch is integrated with similar studies on corn starch. Results are reported under "Corn Utilization," RPA 406, Section A.

B. Microbiology and Toxicology

Research on grain sorghum is integrated with related investigations on corn. Results are reported under "Corn Utilization," RPA 406, Section C.

C. Technology--Process and Product Development

1. Processing studies. In studies on gelatinizing endosperm fractions from corn, sorghum, barley, wheat, rye, and oats by extrusion-, roll-, or direct steam-cooking, the cereals responded similarly except for oats in water absorption and water solubility properties. Highest cold paste viscosities were obtained from corn, sorghum, and barley; lowest from oats. Cooked paste viscosities were highest with barley and oats. Processes were developed for separating endosperm flour and grit fractions from grain sorghum which was first subjected to wet-peeling. Adequate germ recovery from corn was obtained by wet procedures after steeping for only 4 hours at 150° F.

Excellent instant CSM-type products have been developed based on corn and sorghum; similar products with good properties were prepared using oats, wheat, rye, and barley. Stability of ascorbic acid in CSM was satisfactory when stored 6 months at 45° C. at moisture of 8.0 percent or less. Additional studies on CSM involving ingredients, alternative ingredients, and storage stability indicate that present specifications ensure uniformity in composition and in nutritive and use properties. A mixed infant cereal with improved nutrient composition over present commercial products has been developed using oat, corn, wheat, and soy flours with vitamins and minerals. Tentative specifications for USDA purchase have been prepared for instant CSM for gruels and for mixed infant cereal.

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL
PRODUCTS FROM FIELD CROPS

Industrial Products

Research in this category is integrated with similar studies on corn. Results are reported under "Corn Utilization," RPA 407, Industrial Products.

RPA 702 - PROTECT FOOD SUPPLIES FROM HARMFUL MICROORGANISMS
AND NATURALLY OCCURRING TOXINS

Research on aflatoxin and other microbiological and toxicological studies relevant to grain sorghum utilization are reported under "Corn Utilization," RPA 702.

RPA 901 - ALLEVIATE SOIL, WATER AND AIR POLLUTION

Research in this category on grain sorghum is integrated with related investigations on corn. Results are reported under "Corn Utilization," RPA 901.

Publications - USDA and Cooperative Programs

RPA 406 - NEW AND IMPROVED FOOD PRODUCTS FROM FIELD CROPS

Chemical Composition and Physical Properties

Sastry, L. V. S., and Virupaksha, T. K. (Indian Institute of Science, Bangalore, India). 1967. Disc electrophoresis of sorghum seed proteins in polyacrylamide gels. Anal. Biochem. 19(3), pp. 505-513.*

Shoup, F. K., Deyoe, C. W., Campbell, J., and Parrish, D. B. (Kansas State University, Manhattan, Kansas). 1969. Amino acid composition and nutritional value of milled sorghum grain products. Cereal Chem. 46(2), pp. 164-171.

*Research supported by PL 480 funds.

Virupaksha, T. K., and Sastry, L. V. S. (Indian Institute of Science, Bangalore, India). 1968. Studies on the protein content and amino acid composition of some varieties of grain sorghum. J. Agr. Food Chem. 16(2), pp. 199-203.*

Technology--Process and Product Development

Anderson, R. A. 1969. Research on improved methods of milling sorghum grain. Proc. 6th Grain Sorghum Research and Utilization Conference, Amarillo, Texas, March 5-7, 1969, pp. 32-36.

Blessin, C. W., Anderson, R. A., and Inglett, G. E. 1968. Current grain sorghum research. Sorghum Newsletter 11, pp. 23-25.

*Research supported by PL 480 funds.

SOYBEAN UTILIZATION

USDA and Cooperative Programs

Problem Area		Scientist Man-Years FY 1969 (Estimated)		
No.	Title and Activity	Intra- mural	Extra- mural	Total
406	New and Improved Food Products from Field Crops			
	Chemical Composition and Physical Properties	8.9	1.4	10.3
	Flavor	8.5		8.5
	Color, Texture and Other Quality Factors	3.9		3.9
	Microbiology and Toxicology	0.2		0.2
	Technology--Process and Product Development	2.2		2.2
	Subtotal	23.7	1.4	25.1
407	New and Improved Feed, Textile, and Industrial Products from Field Crops			
	Industrial Products			
	Chemical and Physical Investigations to Improve Products	4.0	0.6	4.6
	*Technology--Process and Product Development		1.3	1.3
	Subtotal	4.0	1.9	5.9
601	Expansion of Foreign Markets for U.S. Products			
	Technology--Process and Product Development	2.6		2.6
	Subtotal	2.6		2.6
901	Alleviate Soil, Water and Air Pollution Technology--Process and Product Development	1.5		1.5
	Subtotal	1.5		1.5
	Total	31.8	3.3	35.1

Domestic program supplemented by PL 480 funds in 4 countries totaling 547,366 U.S. dollars equivalent (India, Israel, Japan, United Kingdom).

*Contract negotiated at end of fiscal year covering research on nylon-9 from soybean oil; no report in text.

Problems and Objectives

The worldwide need for dietary protein and for food fats poses a problem that urgently demands solution. U.S. soybeans could play a dominant role in alleviating these shortages in developing countries and elsewhere around the world, if soybean protein products can be successfully used in food products tailored to meet the various nutritional and palatability requirements and if opportunities for foreign utilization of soybean oil can be increased by development of inexpensive processes for insuring flavor stability. Solution of these problems would also contribute to increased domestic use of soybean food products and soybean oil. Nonfood usage of soybean oil has rather consistently accounted for about 10 percent of domestic disappearance. In view of the increasing production of soybeans and soybean oil, maintenance of nonfood markets requires continuing effort to develop products that can compete with synthetics derived from nonagricultural sources.

Major objectives of current research are to develop and evaluate alternate ways for:

1. Producing high-protein foods based on full-fat soybean flour.
2. Removing objectionable flavors and other factors from soy products.
3. Increasing flavor stability of soybean oil.
4. Improving refining processes for soybean oil by reducing cost and associated water pollution.
5. Converting soybean oil to competitive industrial chemical products.

Progress - USDA and Cooperative Programs

RPA 406 - NEW AND IMPROVED FOOD PRODUCTS FROM FIELD CROPS

A. Chemical Composition and Physical Properties

1. Mass spectrometry of glyceride oils and derivatives. The mass spectrometer provides an invaluable aid in the location of groups on fatty acid chains, in the study of deuterium exchange during hydrogenation, and in isomerization in determining structures such as aflatoxin derivatives. Glyceride structures of fats can now be analyzed rapidly by mass spectrometry with less than milligram samples and in a few minutes to give information comparable to that obtained by countercurrent distribution in a few weeks on multigram samples. The resolution of the magnetic mass spectrometer has been increased from 2,000 to 20,000 by an electrostatic analyzer. Molecular formulas can now be assigned directly from the high accuracy mass number.

Mass spectrometric analysis of headspace gas provides an accurate method for determining the oxygen concentration in the inert gas blanketing, the bottling and storage of soybean and other salad oils.

The intensity of the parent peak of long chain fatty esters is enhanced greatly by a recently developed field ionization source. Intensity doubles for each increase of two carbon atoms to the chain length; practical application for molecular weight determinations await development of a combined field ionization-electron impact source.

2. Basic studies on soybean protein. The effects of soybean variety, meal age, and concentration of 2-mercaptoethanol on water extractability of proteins from defatted soybean meal were studied. Use of 2-mercaptoethanol in the extraction solvent increased protein extractability. Effects of other factors are being analyzed statistically. After chromatographic purification, crystalline soybean trypsin inhibitor serves as a useful reference protein in gel electrophoretic studies of soybean proteins. Soybean meal contains nucleotides which appear to be complexed with pigments. The nucleotide-pigment complex is sensitive to heat, alcohol, and alkali. Genistein has been identified as one of the pigments in the complex. Soybean saponins can be separated into 10 fractions by thin-layer analysis and into 7 fractions by anion-exchange chromatography. Soybean saponins are more complex mixtures than is indicated by previous work.

3. Lipoxidase studies. The products of soybean lipoxidase-catalyzed oxidation of linoleic acid were reexamined, and the mechanism of the reaction was studied. Previously published reports indicated keto-stearate as well as hydroxystearate in the lipoxidized and catalytically hydrogenated products. We have now established that keto-stearate is an artifact produced by the catalytic hydrogenation.

When the double bond system of linoleic acid is conjugated by soybean lipoxidase, no exchange of protons with the solvent occurs. This is in marked contrast to alkaline conjugation.

Soybean lipoxidase is able to oxidize 12,15-octadecadienoic acid, an unnatural isomer of linoleic acid produced by partial hydrogenation of linolenic acid. The 12,15 diene is apparently oxidized at a much slower rate than the naturally occurring 9,12 diene.

The proportion of 13-hydroperoxy to 9-hydroperoxy dienes in the products of lipoxidase-catalyzed oxidation of linoleic acid appears to increase over the course of the incubation.

Preliminary experiments indicate that soybean lipoxidase can oxidize unsaturated acyl chains of solubilized soybean phosphatides in vitro. This may be an important reaction in vivo.

4. Flatulence factor of soybeans. At the University of Illinois, Urbana, Illinois, under grant research, surgically treated dogs were given a flatus-producing soybean diet to determine its effect on the type and amount of microorganisms in the intestine in relation to normal basal diets. The intestinal gas-producing anaerobic bacteria increased greatly in relation to the six bacterial groups tested. Phenolic acids, such as ferulic and syringic, will temporarily inhibit growth and gas-producing ability of these anaerobes. Certain amino acids are also effective inhibitors of gas. In-vitro tests have shown that flatus activity of soy flour and textured vegetable proteins is reduced greatly after sterilization, whereas toasting was without effect. Twenty-three soybean varieties and strains differed widely in their ability to cause gas in vitro. There was a seven- to eightfold difference in amount of gas produced with certain of the soybean samples. A method has been developed to record intestinal activity and histological changes in the gastrointestinal tract during in-vivo ingestion of a soybean diet.

5. Antinutritional factors in soybeans. Fifty-two samples of commercial and 56 samples of noncommercial soybean varieties were analyzed for moisture, fat, protein, amino acid composition, antitryptic and hemagglutinating activity. The samples were classified as low, medium, and high in antitryptic and hemagglutinating activities. Samples representative of the three categories were selected for future feeding studies. Natural and synthetic substrates have been evaluated for measurement of trypsin inhibitor activity in soybeans.

This research is being conducted under a grant to the University of Minnesota, Minneapolis, Minnesota.

6. Minor constituents of soybeans. These studies are being conducted under PL 480 grants at foreign institutions. At Kagawa University, Takamatsu, Japan, an examination of 20 strains of Escherichia coli yielded one strain which produced a high activity of α -galactosidase, an enzyme capable of hydrolyzing the α -galactoside linkages of soybean oligosaccharides. The bacterial enzyme was purified 142-fold by precipitation with sodium sulfate and adsorption and desorption on calcium phosphate gel. The purified enzyme has a pH optimum of 6.8 and optimum temperature of 37° C. The effects of buffer ions and other salts on enzyme activity were studied, as well as the rates of hydrolysis of different substrates containing the α -galactoside linkage. Dilute solutions of the enzyme lost activity rapidly, but concentrated solutions were much more stable. Other intestinal bacteria are being examined for α -galactosidase activity.

In studies completed during the year at the Weizmann Institute of Science, Rehovoth, Israel, the structure of a glycopeptide, isolated from purified soybean hemagglutinin, has been studied by enzymatic and chemical methods. The N-acetylglucosamine residues are located in the interior of the glycopeptide and the mode of linkage has been partially determined. The mannose residues are arranged into three distinct regions separated by N-acetylglucosamine residues. A red-colored band observed during chromatographic

purification of soybean hemagglutinin has been identified as cytochrome c. Soybean cytochrome c was purified and characterized. The purified enzyme was homogeneous on polyacrylamide gel electrophoresis; its spectral properties and amino acid composition closely resemble those of cytochrome c from wheat germ.

Isolation and characterization of soybean sterols extracted from flaked beans with ligroin and alcohol-benzene was undertaken at the Hebrew University of Jerusalem, Jerusalem, Israel, under a PL 480 grant. Oil obtained from the first extraction made with ligroin contained mainly sterol glycosides (0.457% sterols), and the ethanol-benzene extracted oil (0.013% oil) contained equal amounts of sterol esters and glycosides (0.348% sterols). The amount of free sterols is very small (0.015%). Efforts are being made to obtain in pure form all soybean sterols and triterpenes by preparative gas chromatography, column, and "dry-column" chromatography. Cholesterol derivatives and isomers do not possess the biological availability for insect larvae (Dermestes maculatus) which cholesterol shows. For normal growth and metamorphosis it was found that 0.03 percent of cholesterol is required in the diet. A study on the utilization of the C28 and C29 sterols and their derivatives by various insects will be undertaken when satisfactory methods of sterol purification are developed. Four derivatives of stigmasterol have been synthesized and are under biological test.

At the University of Tokyo, Tokyo, Japan, a magnesium silicate (Florisil) column chromatographic procedure was developed to determine the various classes of soybean sterols: free sterols, sterol esters, and steryl glucosides. A previously unknown form of soybean sterol was discovered and shown to be acylated steryl glucosides. A total of 39 samples of soybeans was analyzed including 19 U.S. and 5 Japanese varieties. Total sterol contents ranged from 0.11-0.19 percent. The range of distribution of the sterol forms was 25-57 percent free, 7-23 percent esters, 8-33 percent steryl glucosides, and 17-36 percent acylated steryl glucosides. These studies were completed during the year.

7. Triglyceride syntheses. Under a PL 480 grant to the University of Bombay, Bombay, India, model compounds intermediate to new triglycerides have been prepared and their purity determined by thin-layer chromatography. Monoglycerides, 1-palmitin, 1-stearin, and 1-elaidin were prepared in 0.5-kilogram quantities from 1,2-isopropylidene glycerol-3-acetin by ester interchange. Diglycerides, 1,3-dipalmitin, 1,3-distearin and 1,-stearo-3-palmitin, 1-elaido-3-stearin and 1-elaido-3-palmitin were prepared and are being purified. The grantee is preparing various other pure fatty acids and their mono-, di-, and triglycerides of known structure.

8. Physiological effects of soybean inhibitors. At Baylor University, Houston, Texas, dogs with pancreatic and gastric cannulae and Pavlov pouches are undergoing conditioning tests prior to the beginning of feeding experiments to determine physiological effects of various soybean factors. To

evaluate the results of the feeding trials, enzyme methods for ribonuclease, desoxyribonuclease, α -amylase, chymotrypsin, trypsin, and lipase were established. Data for establishing normal bicarbonate, enzyme, and acid levels are being obtained.

B. Flavor

1. Selective hydrogenation--heterogeneous catalysis. Research is continuing on the use of copper catalysts to selectively hydrogenate linolenate in soybean oils and on the products formed. Unlike nickel catalysts, copper hydrogenates only double bonds which are conjugated or conjugatable (methylene interrupted). Partial hydrogenation of linolenate produces a variety of isomeric esters and the monoenes have double bonds from 3rd to 17th carbon. Competitive hydrogenations of isomeric methyl octadecenoates with nickel, platinum, or palladium show that oleate is reduced more rapidly than elaidate; the cis 15 isomer is reduced more rapidly than oleate; rates for the 6 and 12 isomers vary with the catalyst. A general digital computer program has been developed to determine rate constants for complex reactions like hydrogenations. Alkali isomerization of linoleate and linolenate has been shown by deuterium and tritium tracers to proceed by a carbanion mechanism. Gas chromatographic equivalent chain length data has been obtained for identification of fatty methyl esters. Copper catalysts reduce triple bonds to cis double bonds. A method has been developed for dual label low-level radioactive measurements in gas chromatography fractions.

2. Evaluation of edible soybean oil products. Storage tests indicated that commercial salad oils packaged under nitrogen had far greater stability than oils not protected by an inert atmosphere. Hydrogenated-winterized soybean salad oil when protected by nitrogen showed only a slight decrease in flavor score after storage for 1 year at 100° F. For extended shelf-life, packaging under inert atmosphere appears to be just as or more important than hydrogenation toward improving the keeping quality of soybean oil. Longer shelf-life is indicated for all salad oils when packaged in the complete absence of oxygen.

The stability of high iodine value sunflower salad oils was improved by the addition of antioxidant mixtures containing metal sequestrants. A 20-hour fry test comparing sunflower oil to a cottonseed-corn oil mixture demonstrated that potato chips fried in sunflower oil received higher flavor scores than potato chips fried in the oil mixture in each evaluation over a 4-week storage period.

Oxidative dimer formation in fatty esters was followed by radio tracer experiments. Results of oxidation studies indicate that the dimer is formed equally from the hydroperoxide and the unsaturated ester.

3. Flavor components of soybean meal and protein. Previous studies showed that hexane:methanol (75:24 v/v) azeotrope extraction of hexane-defatted meals reduced the intensity of the undesirable flavors characteristic of raw

soybean meals. A series of meals has been extracted for varying times to produce products that will be evaluated by a taste panel and that will be used as a source of protein isolates.

Since Molsin, a commercially available enzyme preparation, was found to be effective in removing objectionable flavors from soybean protein products in research at the University of Tokyo, Tokyo, Japan, further studies were made with it. Aspergillopeptidase-A appears to be an endopeptidase which participates in enzymatic action to remove beany and bitter flavors. Molsin also contains a carboxypeptidase, an exopeptidase that contributes to the removal of beany-bitter flavors. Bitterness that results with peptic hydrolysis of soybean protein at 37° C. was ascribed partly to a series of diffusible di-, tri-, and tetrapeptides, such as glycyl-leucine, leucyl-phenylalanine, and seryl-lysyl-glycyl leucine. A nondiffusible peptide from the peptic hydrolysis and containing 24 amino acids also proved to be bitter. Treatment with carboxypeptidase-A lowered the bitterness of both diffusible and nondiffusible peptides. Bitter peptides in the diffusible fraction were ninhydrin negative because peptic hydrolysis forms N-terminal pyrrolidone carboxyl residues by intramolecular cyclization. This research is being conducted under a PL 480 grant.

C. Color, Texture and Other Quality Factors

1. Minor constituents of soybeans. Extracting 99.8 percent of the oil from full-fat soybean flakes with pentane-hexane removed little of the green-beany bitter flavor. This crude oil with paraffin-like, vegetable oil flavors did not develop any flavors on storage. Further extraction of defatted flakes with hexane-absolute ethanol azeotrope (79:21) removed the rest of the oil and residual lipids, together with most of the flavor. Direct extraction of full-fat flakes with the azeotrope solvent was also effective in extracting most of the lipids and flavor. Since trace amounts of hydroperoxide were detected in some lipid extracts and volatile carbonyl compounds were isolated, a thiobarbituric acid assay (TBA) was used to determine extent of lipid oxidation. A distillation procedure was used to obtain the TBA-reactive substances which were measured at 532 mμ. The absorbance values were converted to milligrams malonaldehyde per 1,000 grams sample. These values are reported as "TBA numbers." Soybean oil and azeotrope extract of defatted flakes had TBA number of 6 and 34, respectively. Each lipid fraction accounted for only 10 percent of the total TBA number of full-fat flakes. Other lipid extracts had TBA numbers as high as 67 but had no objectionable flavor. The extent of lipid oxidation, as measured by TBA assay, therefore, did not correlate with organoleptic evaluations.

2. Color reversion of edible soybean oil. At the Sugiyama Chemical Research Institute, Tokyo, Japan, under a PL 480 grant, studies on purified fatty esters, both saturated and unsaturated, and paraffin oil were made with various combinations of tocopherol isomers, tocored and tocopherol dimers. Tentative conclusions were drawn that tocored is converted in part to a coupling product with hydroperoxides under oxidizing conditions, and that

this product is part of the chain propagation mechanism of autoxidation. On breakdown, the product again releases tocopherol. The proposed oxidation reaction sequence is that γ -tocopherol is converted to tocopherol dimer, which in turn is oxidized to tocopherol, and further oxidation of tocopherol gives unknown compounds. Lipid peroxides supposedly enter into each of these reactions, and γ -tocopherol inhibits the oxidation of dimer and tocopherol. Similar reactions were reported to occur in soybean oil.

D. Microbiology and Toxicology

1. U.S. soybeans for making tofu. Studies under a PL 480 grant to the Tokyo University of Education, Tokyo, Japan, to develop an easily digestible fermented protein food having good flavor, texture, and superior keeping quality were completed during the year. The soybean protein food prepared was a cheeselike product. Normal dairy cheesemaking procedures were unsatisfactory, but the process developed consisted of the following steps: (1) Use salt agglutinant to make a curd; (2) ripen the curd with added proteolytic enzyme; and (3) add a lactic acid bacteria starter to improve the flavor. The optimum conditions were found to be the addition of calcium sulfate at the level of 0.03-0.04 N to soy milk at 70° C. The most suitable commercial enzyme was papain and the ideal ripening temperature was 17.5° C. for 3 weeks. The product had good flavor and texture. The nutritional value of the product determined by rat-feeding experiments was better than that of traditional soybean foods. It had a PER of 2.7 as compared to 2.2 for soybean curd.

Data from completed research under a PL 480 grant to the Japan Tofu Association, Tokyo, Japan, for commercial, commercial plus pilot-plant, and laboratory tests on the quality of tofu from different soybeans have been analyzed statistically. Rankings made in this evaluation confirm previous rankings. Hawkeye stands out as the best variety. The rankings in commercial tests were: Hawkeye (Illinois), Harosoy (Illinois), Kanrich (Illinois), Hawkeye (Iowa), Chippewa-64 (Iowa), Hark (Illinois), Amsoy (Illinois), Lee (Sample 2-Mississippi), and Traverse (Minnesota). Rankings in commercial and pilot tests by variety were: Hawkeye, Hark, Harosoy, Chippewa, Lee, Amsoy, Corsoy, Kanrich, Traverse, and Semmes. No statistically significant differences were found except in the laboratory tests. Of the 29 samples tested in the laboratory, tofu quality from Hawkeye was significantly better than 15 of the 29 samples tested. Rankings (laboratory) of the better samples were: Hawkeye (Illinois and Iowa), Clay (Minnesota) and the following all tied were Kanrich (Illinois), Chippewa-64 (Iowa), Harosoy-63 (Illinois), Hark (Illinois), Chippewa (Minnesota), and Provar (Illinois). Phosphorus content of soybean milk was found to affect yield and quality of tofu in coagulation studies with calcium salts. Protein-phosphorus ratio appears to be involved.

2. Studies on miso and shoyu. Investigations to produce stable diploids of Saccharomyces rouxii, the yeast used in the production of the soybean foods, shoyu and miso, have been conducted under a PL 480 grant at the Noda Institute for Scientific Research, Noda-Shi, Chiba-ken, Japan. Many

cultivated strains (2,300) were investigated and stable diploids were produced possessing characteristics of each parent. The flavoring compounds produced in varying amounts by S. rouxii are iso-butylalcohol, iso-amylalcohol, 2-phenylethanol, ethyllactate, acetic acid, acetoin, and esters. Methods for carrying out small-scale shoyu fermentations to evaluate the hundreds of isolates were developed. Studies of selected diploids were confirmed in small- and medium-sized fermentations (300 liters). The feasibility of using hybrids of S. rouxii which could be kept in the diploid condition was conclusively established. A complicating factor was the discovery that strains of a slower growing yeast, Torulopsis, was important in flavor formation in shoyu. Species of this yeast do not mate with S. rouxii and no hybrids could be produced. These studies were completed during the year.

In studies under controlled conditions at the Japan Shoyu Research Institute, Tokyo, Japan, it was determined that yield and quality of shoyu (soya sauce) by fermentation procedures depends on the nitrogen content of the soybean protein product used as starting material. Thus, alcohol-washed soybean meal gave higher yields than American soybeans defatted by Japanese methods which, in turn, were higher than Japanese defatted soybeans. American grits and soybean meal and Japanese soybean meal (smaller than 8 mesh) did not give yields as high as expected based on nitrogen content. Clods tend to form and other problems occur. Japanese meal could be used, but it was no better than defatted soybeans. American grits and meal rated lower, and they will not be used unless clod formation in steam treatment and preparation of mashes and moromi can be overcome. Specially prepared U.S. soybean meal of uniform size gave the best results of any meal as evaluated at the end of 3 months of fermentation. With the exception of the specially prepared U.S. meal (results incomplete), alcohol-washed soybean meal gave the best results but flavor was not better (testing was limited because of destruction of meal in shipment). These studies are being conducted under a PL 480 grant.

E. Technology--Process and Product Development

1. Pilot-scale selective hydrogenation. In studies on the selective hydrogenation of soybean oil with copper-chromite catalysts, no practical reuse of the catalyst was achieved although several methods of treating used catalyst were examined. Certain recently improved or developed commercial Cu-Cr catalysts used in pilot-plant hydrogenation tests were found to have a high degree of activity and selectivity for the hydrogenation of soybean oil. NU studies which showed the proper method of heat treating commercial catalysts for maximum activity have contributed substantially to this improvement which should have a significant impact on promoting industrial acceptance of these highly selective catalysts. Extensive hydrogenation-winterization studies on soybean oil using an improved laboratory method of winterization have established comparative yields of winterized oils after hydrogenation with Cu-Cr and with Ni catalysts to various linolenate levels. Substantially higher yields of winterized oil at a low linolenate content were obtained with the Cu-Cr catalyst. Flavor stability data indicate little improvement

in soybean oil is obtained unless the linolenate is substantially removed and this appears to be practical by partial hydrogenation with Cu-Cr catalyst. The cost of using this catalyst may be slightly higher than for Ni catalyst unless economic means can be found to reuse the catalyst.

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL
PRODUCTS FROM FIELD CROPS

Industrial Products

A. Chemical and Physical Investigations to Improve Products

1. Aldehyde oil derivatives. In the synthesis of nylon-9 from soybean oil, studies were continued on aqueous ozonolysis, hydrogenation of the ozonolysis products, synthesis of the amino acid, and on the removal of chromophoric agents that discolor the polymer. In aqueous emulsions, water reacts better in water-in-oil than in oil-in-water dispersions. Palladium black is a good catalyst for hydrogenation under 150-300 pounds of hydrogen pressure but the alkyl soyate must be refined with alumina to remove catalyst poisons. Polymer discoloration is caused by trace amounts of nickel (150 p.p.m.) and possibly other chromogens introduced during recrystallization. Molecular weights of from 30,000 to 78,000 are easily achieved with purified amino acid; molecular weights of 8,000 to 10,000 only are obtained from crude amino acid. Exploratory work with aldehyde oil indicates that intermediate alkyl soyate formation may not be necessary. Thermally stable stationary phases for GLC columns having good resolution for both polar and nonpolar substances were prepared with poly(ester-acetals) on acidic supports. Several alcohols were prepared from linseed ozonolysis products: methyl 9-hydroxynonanoate in greater than 90 percent, 1,9-nonanediol in 80 percent, and 1,3-propanediol (as 1,3-dioxan) in 35 percent yields.

2. Homogeneous catalytic hydrogenation. Arene chromium carbonyl complexes are effective soluble catalysts for the hydrogenation of vegetable oils. These catalysts are selective in converting polyunsaturates into cis-monounsaturates without formation of saturates. Liquid stable salad oils were prepared from soybean and safflower oils directly without winterization. Liquid fatty acid products approximate the composition of commercial oleic acid. Methyl benzoate-Cr(CO)₃ and Cr(CO)₆ also catalyze the conjugation of polyunsaturated fats. Soybean, safflower, and linseed oils were conjugated to the extent of 60-70 percent.

The mechanism of hydrogenation and conjugation were studied by following kinetics and by using deuterium as a tracer. The unique selectivity of the chromium carbonyl catalysts can be explained by their ability to promote 1,4-addition of hydrogen on intermediate conjugated dienes.

In studies conducted under contract at the University of Illinois, Urbana, Illinois, platinum-tin complexes catalyze selectively the hydrogenation of polyunsaturates of soybean methyl esters into monounsaturates without

significant increase in stearate. The activity of these catalysts was varied by changes in the substituents of the ligand. Various new and known complexes of palladium, cobalt, iridium, osmium, ruthenium, molybdenum, and tungsten were active as hydrogenation catalysts for soybean methyl esters. The complexes of palladium, molybdenum, and tungsten were as selective as the platinum complexes but less active. The complexes of cobalt, iridium, osmium, and ruthenium were much more active but less selective in promoting the formation of stearate. Basic knowledge acquired in homogeneous catalysis should permit us to fashion more precisely tailor-made catalysts of desired selectivity.

3. Isomerization studies. Studies at the University of London, London, England, have disclosed that alkali isomerization of fatty acids of the same chain length and containing double bonds close to the carboxyl group gives mixtures of approximately the same composition. Trans-hex-2-enoic acid, on treatment with 5 N aqueous potassium hydroxide at 90° C., changes gradually to contain 64 percent of original acid, 26 percent of trans-hex-3-enoic acid, 8 percent cis-hex-3-enoic acid, and 2 percent of cis-hex-2-enoic acid. Trans-hex-3-enoic acid gave 64 percent, trans-2; 27 percent, trans-3; 6 percent, cis-3; 3 percent, cis-2; whereas the cis-hex-2-enoic acid gave 70 percent, trans-2; 23 percent, trans-3; 6 percent, cis-3; and 1 percent of cis-2. Syntheses of stereoisomeric tridecadienes (5-8 and 5-7) are in progress. This research is being conducted under a PL 480 grant.

4. New derivatives from soybean and linseed oils. Ozonolysis of the ethylene adduct of methyl linoleate followed by reduction or thermal decomposition gives excellent yields of the ester dialdehyde or diester acid, respectively. The main byproduct (17%) formed during thermal decomposition is methyl 9(12)-carbomethoxy stearate.

Preliminary viscosity measurements indicate that the mixture of di- and triester from the thermal decomposition may be a suitable base for the formulation of new jet lubricants. Peracid oxidation of the propylene adduct of methyl linoleate gives the hydroxyformoxy and glycol derivatives.

Several new cyclic fatty acid derivatives have shown juvenile hormone activity at 10-100 µg. levels. An alkyd made from a fluoro-cyclic fatty acid and exposed outdoors for 19 months shows promise as a durable clear lacquer.

B. Microbiology and Fermentation

1. Microbial modification of fatty acids. Three new hydroxy fatty acids have been prepared by utilizing the microbial enzymatic hydrolase activity of two bacteria of the genus Pseudomonas. Thus, linoleic acid was converted to 10-hydroxy-cis-12-octadecenoic acid, linolenic acid formed 10-hydroxy-cis-cis-12,15-octadecadienoic acid, and ricinoleic acid was hydrated to 10,12-dihydroxystearic acid. All three products are optically active; the mono- and di-unsaturated products are moderately levorotatory, while the dihydroxy acid shows low levorotation. Several other fatty acids and one

fatty alcohol having 9,10-double bonds were not hydrated to hydroxy derivatives. A new amino fatty acid, 10-aminostearic acid, was prepared from 10-hydroxystearic acid; intermolecular amidation of this amino acid produced a polyamide. The polymer can form fibers when hot but is a translucent solid at room temperature. This work has been terminated.

2. Lipolytic enzymes. During the past year at the University of Baroda, Baroda, India, the lipase produced by the best yielding strain of Pseudomonas was purified 90-fold by a combination of $(\text{NH}_4)_2\text{SO}_4$ precipitation and Sephadex gel filtration. The purified lipase showed activity against triglycerides and ethyl esters though the relative activity of the pure lipase was greater for triglycerides than for esters when compared to the crude enzyme. This may indicate that the two functions are due to different enzymes. The bacterial lipase was found to be activated by Ca^{++} . Other divalent cations such as Cu^{++} , Zn^{++} , Hg^{++} , and Fe^{++} inhibited lipase activity. This lipase was found to also catalyze the reverse reaction and form glycerides from oleic acid and glycerol. Mono-, di-, and triolein were found in the reaction mixture. This latter capability might be useful in preparation of unique glycerides. These studies are being conducted under a PL 480 grant.

RPA 601 - EXPANSION OF FOREIGN MARKETS FOR U.S. PRODUCTS

A. Technology--Process and Product Development

1. Full-fat soybean flour. Extrusion-cooking technology for converting soybeans to nutritious high-protein flour is being developed. Conditions of operation have been optimized by mathematical analysis to guide a processor in obtaining the best nutritional value, flavor, and storage stability. Lipoxidase enzyme was found to initiate lipid oxidation prior and during extrusion when activated by moisture. A method of lipoxidase inactivation by dry-heating was developed as a preconditioning step prior to extrusion. Long-term storage tests proved this step to be essential for obtaining high product quality. Subsequent studies showed that the presence of chemical additives during extrusion cooking influenced the removal of growth inhibitors and affected flavor, storage stability, and physical properties. Studies on the utilization of the full-fat soy flour in food products have been initiated. A soybean-base beverage powder that disperses in water to give a nutritious high-quality drink has been prepared.

RPA 901 - ALLEVIATE SOIL, WATER AND AIR POLLUTION

A. Technology--Process and Product Development

1. Refining soybean oil. The removal of soap from alkali-refined, unwashed soybean oil by a process in which a cation-exchange resin is employed, was investigated by batch and continuous methods. Water used to wash refined soybean oil and then treated with a hydrogen form cation-exchange resin could be reused to wash additional oil with no reduction in efficiency. By

successful reuse of the wash water, it is believed the pollution problem in vegetable oil refining caused by the deposit of soapy wash water into sewer systems or streams can be eliminated. The sodium level in a resin-recycle washed soybean oil was less than 0.5 p.p.m. which is comparable to that of a conventionally washed and bleached soybean oil. Flavor scores of bleached resin-recycle washed soybean oil were equal to or better than those of conventionally washed and bleached oil after 7 days' storage at 60° C. Soybean oil washed by the resin-recycle method but not bleached was shown to hydrogenate readily. A preliminary cost estimate showed the processing cost to be \$0.06/cwt. of oil processed. A rapid, reliable flame photometric method of analysis for sodium content of soybean oil in the parts-per-billion range was developed.

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RPA 406 - NEW AND IMPROVED FOOD PRODUCTS FROM FIELD CROPS

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RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL PRODUCTS FROM FIELD CROPS

Industrial Products

Chemical and Physical Investigations to Improve Products

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FLAX UTILIZATION

USDA and Cooperative Programs

		Scientist Man-Years		
Problem Area		FY 1969 (Estimated)		
No. :	Title and Activity	Intra- :	Extra- :	Total
:	:	mural :	mural :	:
:	:	:	:	:
407 :	New and Improved Feed, Textile and Indus-	:	:	:
:	trial Products from Field Crops	:	:	:
:	<u>Industrial Products</u>	:	:	:
:	Chemical Composition, Physical	:	:	:
:	Properties and Structure	3.5 :	:	3.5
:	Chemical and Physical Investigations	:	:	:
:	to Improve Products	10.3 :	0.4 :	10.7
:	Technology--Process and Product	:	:	:
:	Development	0.4 :	0.4 :	0.8
:	Total	14.2	0.8	15.0

Domestic program supplemented by PL 480 funds in 2 countries totaling 50,682 U.S. dollars equivalent (India, United Kingdom)

Problems and Objectives

Traditional markets for linseed oil, the major drying oil produced and used in the United States, are threatened by widespread use of synthetic products derived from nonagricultural sources. Recently, annual domestic use of linseed oil has declined to 276 million pounds from a postwar high of over 700 million pounds in 1950. This decrease came primarily by displacement by synthetic materials capable of better performance, particularly in coatings. To restore the level of use of linseed oil, new or expanded markets are urgently needed. The most promising route to achievement of this goal is development of improved protective coating products that can compete with synthetics. Other new outlets can be realized by chemical modification of linseed oil to obtain materials that will find applications in the multibillion-pound annual market for products of the organic chemical industry.

Major objectives of current research are to develop and evaluate alternative ways for:

1. Improving durability of linseed emulsion paints.
2. Establishing economically feasible methods of curing and/or protecting concrete by use of linseed oil.
3. Converting linseed oil by economical processes to products that can compete in the industrial chemical market.

Progress - USDA and Cooperative Programs

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL
PRODUCTS FROM FIELD CROPS

Industrial Products

A. Chemical Composition, Physical Properties and Structure

1. Linseed oil films and emulsions. Swelling of linseed oil paints by water can be reduced greatly by surface treating the pigments prior to incorporation. Treatment of zinc oxide with an organic phosphate and titanium dioxide with an alkyl quaternary ammonium salt produces pigments with very hydrophobic surfaces that do not react strongly with each other or with linseed oil under the influence of moisture. Incorporation of these coated pigments results in decreased swelling and improved tensile properties. Measuring the sedimentation volume of untreated and treated pigments in a series of liquids has resulted in a new method to determine the reactivity and related properties of such pigments. Sedimentation volumes have been related to the solubility parameters of the liquids employed.

Glossy films from linseed oil emulsion paints containing oil-coated pigments showed essentially the same surface texture as films from solvent-based

coatings when viewed under a scanning electron microscope (SEM) whereas films of commercial latex coatings show a very rough texture. The surface texture is directly related to the degree of light reflection needed for glossy appearance. SEM studies have also been shown to be valuable to determine compatibility of linseed oil and latex vehicles. Recently more linseed oil is being used in latex paints to improve adhesion and other properties. We have shown that linseed oil can be blended with polybutadiene-styrene and acrylic latexes to give films with new surface and bulk properties. Blending of linseed oil emulsion with polyvinylacetate emulsion results in films in which patches of linseed oil and latex can be recognized easily. This information will have to be correlated with adhesion studies to be significant for paint formulation. Linseed oil solutions and emulsions penetrate concrete previously cured with commercial wax and resin curing compounds. Penetration through curing compounds occurs to about the same extent as it does into sheet-cured concrete. Significant penetration (1-2 mm.) occurs at 1 and 14 days, less penetration (0.5-1 mm.) at 4 and 7 days, and more penetration (2-3 mm.) at 35 days.

B. Chemical and Physical Investigations to Improve Products

1. New polymers and derivatives for coatings. A Fortran computer program was written to calculate color designations and color differences of urethane-polyesteramide films from spectroreflectometric data. Of our 14 experimental urethane-polyesteramides, 8 linseed and 3 soybean films exposed in the north window and 7 linseed and 4 soybean films stored in the dark were equivalent to or better than films of a commercial urethane-linseed oil after 18 months. Stable emulsions (3.5 μ particle size) of urethane-polyesteramides were prepared by use of an ultrasonic disintegrator. These emulsions coalesce to form clear films when cast on glass plates. Polythiocarbamates have been prepared from tolylene-2,4-diisocyanate and a series of hydrogen-sulfide-treated linseed oils with sulfur contents up to 9 percent. These polymer films with driers air-dry tack free in 7 to 9-1/2 hours. Longer tack-free and dry-to-touch times correlate with decreasing sulfur content. The episulfide of methyl oleate was formed from the photolysis of carbonyl sulfide in a benzene solution of methyl oleate. The episulfide was not isolated, but infrared data and thin-layer chromatography indicate the presence of an episulfide.

In research studies under a cooperative agreement among the Northern Division, the National Flaxseed Processors Association and North Dakota State University, Fargo, North Dakota, aldehyde and epoxy-linseed oils were reacted with hydroxyl-containing polymers, such as low-molecular-weight polyhydroxyethyl-acrylate-derived polymers dried or baked within 1 hour to give glossy, hard, flexible films with good adhesion. Rocker hardnesses of 20 to 60, reverse impact values of 30 to 70 in. lbs. at rupture, and no cracking on a 1/2 in. conical mandrel were achieved. These films and the experimental approach to making them appear promising. Unsaturated fatty acids such as oleic acid were converted with lead tetraacetatecupric acetate to the corresponding terminal olefin, such as 1-octadec-9-ene. Structures of maleic

anhydride-methyl esters of polyunsaturated fatty acid adducts were proposed on the basis of new studies on them showing cyclohexenyl rings. Trichlorosilane derivatives were reacted with epoxy linseed oil to give products that dried to tacky films.

2. Catalytic conjugation. A complex of rhodium chloride and triphenylphosphine has been shown to produce equivalent levels of conjugation (ca. 50%) to those produced by similar platinum complexes when used with stannous chloride. The rhodium complex functions at much lower temperatures and does not require an added cocatalyst. Since it functions as well in chloroform as in alcohols, transesterification products with ester substrates are avoided. Oxygen deactivates this catalyst, a sensitivity not noted with the platinum triphenylphosphine catalysts nor with rhodium chloride.

3. Photochemistry of linseed oil polymers. Research under a grant to the University of Illinois, Chicago, Illinois, has revealed that linseed oil adsorbed on metal oxide substrates reacts exothermally at 150° C. This reaction is essentially an oxidative dehydrogenation with isomerization of isolated double bonds to a conjugated system. Photo-oxidation of linseed oil at lower temperatures is substrate-dependent. The reaction rates increase in the following pigment series: zinc oxide, rutile-titanium dioxide, silica, anatase-titanium oxide. Surface areas of adsorbed model compounds on various substrates have been determined. This research has been terminated.

4. Other relevant research. Studies on linseed oil in this category are integrated with similar investigations on soybean oil. Results are reported under "Soybean Utilization," RPA-407, Industrial Products, Section A.

C. Microbiology and Fermentation

Research in this category is integrated with similar investigations on soybeans. Results are reported under "Soybean Utilization," RPA 407, Industrial Products, Section B.

Publications - USDA and Cooperative Programs

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL
PRODUCTS FROM FIELD CROPS

Industrial Products

Chemical Composition, Physical Properties and Structure

Eissler, R. L. 1968. Novel substrates for preparing free films from paints or polymers. J. Appl. Polym. Sci. 12(8), pp. 1983-1984.

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CRAMBE UTILIZATION

USDA and Cooperative Programs

Problem Area		Scientist Man-Years FY 1969 (Estimated)		
No.	Title and Activity	Intra- mural	Extra- mural	Total
407	New and Improved Feed, Textile and Industrial Products from Field Crops			
	<u>Feed</u>			
	Chemical Composition and Physical Properties	2.6		2.6
	Technology--Process and Product Development	1.8		1.8
	<u>Industrial Products</u>			
	Chemical and Physical Investigations to Improve Products	2.4		2.4
	Technology--Process and Product Development	2.3		2.3
	Total	9.1		9.1

Problems and Objectives

Crambe, a new oilseed crop commercialized in 1965, is the first plant included in the research program on new crops to achieve this status. Crambe seed oil is rich in erucic acid. Several industrial uses already exist for erucic acid as well as for imported rapeseed oil, which formerly was the only source of this acid. However, to insure optimum development of crambe as a new commercial crop, possible markets for crambe oil and erucic acid must be explored and those with the greatest industrial potential must be identified and exploited. In addition, since economic value to the farmer and to industry of any oilseed crop is much greater if the meal left after extraction of the oil can be utilized as a palatable and nutritious feed for animals, suitable processes are required to realize fully the anticipated nutritional qualities and to insure maximum acceptability to different types of animals.

Major objectives of current research are to develop and evaluate alternate ways for:

1. Developing new plastics, resins, and special-purpose plasticizers from crambe oil.
2. Improving protein feed supplements from crambe.

Progress - USDA and Cooperative Programs

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL PRODUCTS FROM FIELD CROPS

Feed

A. Chemical Composition and Physical Properties

1. Crambe enzymes. A study of the nonenzymatic degradation of thioglucosides in the presence of ferrous salts has been extended to 50 species of cruciferous seeds. This survey confirms the previous generalization that only those thioglucosides having a β -hydroxyl group in the aglucon are converted to thionamides by Fe^{+2} ; all other thioglucosides are converted to nitriles.

Soluble crambe thioglucosidase has been purified 14-fold over the crude extract by fractionation on Sephadex G-200. Eighty percent of the enzyme activity elutes at a position corresponding to a molecular weight of 110,000. The enzyme responds specifically to ferrous ion to convert the aglucon of epi-progoitrin to 1-cyano-2(S)-hydroxy-3-butene instead of (R)-goitrin over a range from pH 3.9 to pH 6.7. This effect of ferrous ion in altering the product of the enzyme reaction supports the view that a single enzyme accounts for the varied thioglucoside products found in crambe seed meal. Kinetic constants for crambe thioglucosidase show only minor differences from the corresponding mustard enzyme. Crambe enzyme is not inhibited by 10^{-4} M p-chloromercuribenzoate, but does require a reducing agent for stability. In

the presence of ferrous ion, substrate inhibition appears at high levels of epi-progoitrin. Thioglucosidase enzymes, in autolyzing crambe meal, after a short time lose their ability to convert epi-progoitrin to nitriles and form goitrin instead. Either the Fe^{++} requirement or the relative instability found for the nitrile-forming sequence might be exploited in processing steps to insure improved quality of crambe meal.

2. Conversion products from epi-progoitrin (epi-PG). A new procedure has been developed for accomplishing thioglucoside (glucosinolate) removal by blanching intact seeds to inactivate enzymes, followed by leaching with hot water. The method is applicable to cruciferous seeds that contain glucosinolates which give either volatile or nonvolatile hydrolytic products, with mustard (Brassica juncea) and crambe as examples of seed containing either type of glucosinolate. The aqueous steeps (at 60°-90° C.) remove about 17 percent of the seed weight and account for more than 90 percent of the glucosinolates (4% of seed weight). Essentially none (<0.2%) of the oil is lost. Upon drying, the leached seeds are in their original form, and can be processed for oil and defatted meal by conventional methods. In the laboratory, pretreated flaked seeds, when extracted with petroleum ether, gave good quality oil and meal with 51 to 61 percent crude protein. The meal contained 0.2 to 1.3 percent residual glucosinolates, depending on the number and the temperature of the water-steeping treatments. Research was completed on a GLC procedure adequate for analyzing crambe and rapeseed meals for the presence and amounts of all recognized aglucon degradation products of progoitrin and epi-progoitrin. The method has been applied to a number of cruciferous species, both seed and vegetative parts.

B. Technology--Process and Product Development

1. Crambe protein meal supplements for ruminants. Crambe abyssinica has potential as an oil-producing crop. However, its success depends upon the use that can be made of the byproduct feed meal, which is toxic and growth inhibitory to nonruminant animals unless it is specially treated. Two processes, used individually or combined, are being explored. They are based on either decomposition or removal of the major and possibly only toxin, epi-progoitrin, which comprises 94 percent of the thioglucosides. Cooking crambe meal in the presence of certain chemical additives resulted in the extensive decomposition of epi-progoitrin. Removal of the degraded products by water extraction gave meals that were nontoxic to rats and chicks. A study of amino acid analyses indicated lysine was the only amino acid affected when "basic" cook additives were used. Complete removal of epi-progoitrin from meals cooked without additives, however, was not achieved by the water extraction alone. Assistance was provided an industrial firm to adapt an existing oilseed extraction plant to process mustard seed as an oilseed.

Industrial Products

A. Chemical and Physical Investigations to Improve Products

1. Chemical modification of crambe oil. A total of 33 brassylates and azela-brassylates (i.e., esters from mixed crambe dibasic acids) prepared at NU have now received preliminary evaluation at EU as lubricants. After preparation of larger quantities and more thorough testing of 12 selected esters, including four without β -hydrogen atoms in the alcohol moieties, the best potential lubricants appeared to be bis(2-ethylhexyl) brassylate and bis(2,2-dimethylpentyl) azela-brassylate. The four esters of 2,2-dimethyl alcohols were also tested at EU as plasticizers for poly(vinyl chloride) (PVC), as was ethylene brassylate (which is used as a musk by the perfume industry). The most significant results in the plasticizer evaluations were the exceptional light stability of PVC sheets containing dineopentyl brassylate or dineopentyl azela-brassylate and the rather pleasant odor emanating from the one plasticized with the cyclic ester.

Allyl esters of erucic, high-purity behenic, crude behenic, brassidic, brassylic, azela-brassylic, and mixed crambe fatty acids were prepared by refluxing allyl alcohol and the acids in benzene with p-toluenesulfonic acid as catalyst. Chemical and physical properties were determined for these seven preparations and for the brassidic acid prepared by elaidinization of erucic acid. Gel times for diallyl brassylate were determined under two sets of conditions, and prepolymer of diallyl brassylate was isolated from a third run that had reaction conditions resembling the second. Unlike the commercial diallyl phthalate prepolymers, the brassylate prepolymer is a viscous liquid rather than a solid.

2. Copolymer films. A convenient technique has been worked out for casting internally and externally plasticized poly(vinyl chloride) (PVC) films from tetrahydrofuran solutions on flat glass surfaces. With this technique films were prepared from copolymers of vinyl chloride (VC) and $\text{CH}_2 = \text{CHOOC}(\text{CH}_2)_{11}\text{COOR}$, where R = ethyl, 2-methylpentyl, or n-nonyl. The copolymers from which the films were made varied in amount of plasticizing copolymer incorporated, content of crosslinking agent (divinyl brassylate), and conditions of copolymerization (reaction time and temperature, nature and amount of initiator employed). For comparison, films were also cast from PVC externally plasticized with dioctyl phthalate or homopoly-(2-methylpentyl vinyl brassylate) and from VC copolymerized with commercial cetyl vinyl ether, maleic ester, or vinyl stearate. When enough copolymer was available, several films of different thicknesses were prepared.

B. Technology--Process and Product Development

1. New nylons from high-erucic oils. Both Freon 113 and n-hexane are effective in washing pelargonic from brassylic acid to purify the latter. The amount of polymeric material in crude brassylic acid can be estimated by gel permeation chromatography (GPC), a column technique by which

substances are separated on the basis of difference in molecular sizes. Some success has been achieved in adapting the GPC procedure to preparative scale. IR analysis of an enriched polymer fraction showed absorptions suggestive of ester functionality.

Publications - USDA and Cooperative Programs

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL PRODUCTS FROM FIELD CROPS

Feed

Chemical Composition and Physical Properties

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Industrial Products

Chemical and Physical Investigations to Improve Products

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NEW CROPS UTILIZATION

USDA and Cooperative Programs

Problem Area		Scientist Man-Years FY 1969 (Estimated)		
No.	Title and Activity	Intra- mural	Extra- mural	Total
407	New and Improved Feed, Textile and Industrial Products from Field Crops			
	<u>Feed</u>			
	Chemical Composition and Physical Properties	1.6		
	<u>Industrial Products</u>			
	Chemical Composition, Physical Properties and Structure	7.0		
	Chemical and Physical Investigations to Improve Products	0.8		
	Technology--Process and Product Development	1.1		
	Subtotal	10.5		
702	Protect Food Supplies from Harmful Microorganisms and Naturally Occurring Toxins			
	Microbiology and Toxicology	1.7		
	Subtotal	1.7		
	Total	12.2		

Domestic program supplemented by PL 480 funds in 1 country totaling 66,344 U.S. dollars equivalent (Poland)

Problems and Objectives

Farmers could achieve more economic use of their land if new and profitable crops were available for their choice that would have different end-use patterns from those presently grown. To develop a new crop, three basic steps are involved: (1) survey of wild plants to identify those having both potentially valuable components and promising agronomic potential for use in the United States; (2) detailed physical and chemical studies on components of interest to obtain clues to likely end uses; and (3) selection of the most promising species, followed by additional utilization research to explore uses and demonstrate industrial potential. Close cooperation is needed with plant scientists who provide assistance and advice in acquisition and selection of samples for screening and who have responsibility for subsequent agronomic research such as establishing proper cultural practices and identifying the best strains and varieties.

Major objectives of current research are to develop and evaluate alternate ways to:

1. Identify new plant sources for industrial vegetable oils from among samples collected worldwide.
2. Determine utilization potential of oils and feed meals from selected new oilseeds.
3. Produce marketable rotenoid products from the domestic legume Tephrosia vogelii.
4. Produce papermaking pulp from kenaf.

Progress - USDA and Cooperative Programs

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL PRODUCTS FROM FIELD CROPS

Feed

A. Chemical Composition and Physical Properties

Research in this category is integrated with similar investigations on crambe. Results are reported under "Crambe Utilization," RPA 407, Feed, subheading A.

Industrial Products

A. Chemical Composition, Physical Properties and Structure

1. Screening for new industrial oils. Half of the 848 seed samples received for screening represented species new to our collection. Analyses were performed on 816 seed samples, 274 oil samples from many species, and

221 Brassica oil samples. Analysis of 129 Umbelliferae samples completed our examination of the 275 in our collection. A number of unusual acids were found in new species: lesquerolic, cis-5-C₂₀ and C₂₂, vernolic, parinaric in acetoglycerides, and γ -linolenic. New acids were found in minor amounts in Picramnia sellowii oil (petroselaidic and cis-6 and trans-6-C₁₆) and Stenachaenium macrocephalum (hydroxy dienoic and epoxy acids, some with trans-3 unsaturation). The hydroxy acid in Nerium oleander oil occurs in the acetylated form. Only Briza spicata, among 41 species (mostly grasses) tested, produces oil with major amounts of galactolipids. Traces were found in oil from wheat, oats, B. maxima and Lagurus ovata (a grass). Solids in Vernonia oil appear to be sterol esters and those in sunflower oil are waxes. Analyses of old Crepis seed indicate that helenynolic, hydroxy conjugated dienoic, and epoxy acids can be formed in quantity during storage. Picramnia oil appears to have activity as a sterilant for female boll weevils.

A new ozonolysis procedure was developed and has been found useful for structure determination of acetylenic, eneyne, and hydroxy, conjugated dienoic acids. Analysis of old (up to 40 years) samples of Crepis seed revealed epoxy and conjugated acids in all samples, including C. foetida, fresh samples of which had insignificant amounts of these materials. Reexamination of a sample of C. thomsonii stored at NU at room temperature for only a few years showed that oxygenated acids had formed during storage.

Final studies under a PL 480 grant at the Swedish Seed Association, Svalof, Sweden, showed that of 122 individual, self-fertilized crambe plants, 12 contained 63-64 percent of total C₂₂ acids and 7 had less than 58 percent. The six high-erucic plants had 60-63 percent erucic acid in the oil, essentially the same as the high oils from the 1965 crop. Similarly, 80 plants of Brassica carinata produced no improvement in the maximum erucic acid content. The range, 33-47 percent C₂₂, however, suggests probable success in increasing the erucic content to approach that of crambe. Twenty-five plants of B. napus showed the same range in thioglucoside content as previous groups. Five plants of the Polish variety Bronowski had about 1/10 the amount of thioglucoside usual for summer B. napus, but two were comparable to normal summer rape, presumably because of outcrossing. The amount of p-hydroxybenzyl isothiocyanate in B. hirta meal ranged from 23-40 mg./g. Two groups of crambe plants (139 total) exhibited the same range of oxazolidinethione, 9-17 mg./g. meal, and the same mean, about 13 mg./g. The breeding studies during the year did not result in erucic acid percentages significantly higher than reported earlier for the species under investigation.

2. Characterization of seed oils and component fatty acids. A novel lipid, a hydroxy cyanohydrin diester, has been isolated from the seed oil of Cordia verbenacea. Progress has been made in the characterization of other unusual seed oils, as follows: Erlangea tomentosa (a source of epoxy glycerides), Monnina emarginata (a source of hydroxy and keto acids), Holarrhena dysenterica, Nerium oleander, Teucrium depressum, and Penstemon spectabilis. A unique macrocyclic lactone has been isolated from Monnina emarginata seed

oil. Carbohydrate constituents of kenaf bark and core have been fractionated. The bark contains about 50 percent cellulose, and core is approximately 30 percent of this constituent. Each portion of the kenaf contains lesser amounts of the various fractions of hemicellulose fractions.

Initial studies under a PL 480 grant at the Institute of General Chemistry, Warsaw, Poland, have been literature searches to permit selection of preferred synthetic methods for synthesis of triglycerides of defined structure, and the preparation of reserve amounts of highly purified erucic and oleic acids and of their acid chlorides. Trierucin of 99 percent erucic acid content was synthesized in 30 percent yield by direct acid-catalyzed (p-toluenesulfonic acid) esterification of glycerol with erucic acid. A 50-g. sample of the trierucin was supplied to the Northern Division in accordance with terms of the grant. The trierucin was apparently of good purity since it contained no free acid and had only trace amounts of trans-isomers or of mono- or diglycerides. As intermediates for further syntheses, 1-mono-olein and 2-monoerucin were prepared and purified. Analytical methods are being evaluated for assessing purities of intermediates and products.

3. Rotenoid determinations in Tephrosia vogelii. Considerable automation of the thin-layer densitometric method for determination of rotenoids has been achieved. Variations usable by agronomists and others when speed and simplicity are of prime importance, with possible sacrifice of some precision and accuracy, have also been demonstrated. As in previous years, leaflets of Tephrosia vogelii grown in 1968 contained several times as much total rotenoid in contrast to the other parts. One variety still contained only deguelin and no rotenone throughout the entire plant; another that was rotenone-free in 1966 had lost this property by 1968. Earlier preliminary evidence of successful preparation of a rotenoid-rich fraction by leaf-stem separation has been confirmed and repeated on a larger scale.

B. Technology--Process and Product Development

1. Chemical pulps from kenaf. A first attempt to prepare pulp from green kenaf continuously by the sulfate process in a pilot-plant, horizontal-tube digester resulted in a successful 20-hour run. Similar processing with field-dried kenaf disclosed need for study of equipment design to achieve optimal results. A 500-day storage study on kenaf preserved by ensiling has been completed. Recovery of solids ranged from about 75 percent at 100 days to 50 percent at 500 days. Yields of screened pulps, basis digester charge, increased from an original 38.8 percent to 45-50 percent for washed silages. Strength properties of sulfate pulps from washed silages were comparable to those from high-quality fresh kenaf, but they decreased slightly with increasing age of ensilage. Consumption of cooking chemicals per unit of pulp produced at 100 days is less than or equal to that for the original, and then increases with age of ensilage. Requirements for bleaching chemicals for pulps from washed silages are about 25 percent of those for the original kenaf. Ensiling without additives was effective but acidification at ensiling time contributed to greater economy in chemical utilization. Maximum

benefits were realized at 100 days, but economy in bleaching improved with duration of storage.

RPA 702 - PROTECT FOOD SUPPLIES FROM HARMFUL MICROORGANISMS
AND NATURALLY OCCURRING TOXINS

A. Microbiology and Toxicology

1. Tumor inhibiting substances from plants. Fractionation and biological assay of Cephalotaxus harringtonia alkaloids has progressed to the point where it is apparent that most, if not all, of the antileukemic activity resides in a single alkaloid which has been assigned the name "harringtonine." The structure of harringtonine has been partially elucidated in cooperative research involving a combination of spectroscopic work at the Northern Division and X-ray crystallography at the University of Pittsburgh.

Publications - USDA and Cooperative Programs

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL
PRODUCTS FROM FIELD CROPS

Industrial Products

Chemical Composition, Physical Properties and Structure

- Appelqvist, L. A. (Swedish Seed Association, Svalof, Sweden). 1969. Lipids in Cruciferae. IV. Fatty acid patterns in single seeds and seed populations of various Cruciferae and in different tissues of Brassica napus L. Hereditas 61(1-2), pp. 9-44.*
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- Kleiman, R., Spencer, G. F., and Earle, F. R. 1969. Boron trifluoride as catalyst to prepare methyl esters from oils containing unusual acyl groups. Lipids 4(2), pp. 118-122.
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*Research supported by PL 480 funds.

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Technology--Process and Product Development

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FORAGE UTILIZATION (NORTHERN REGION)

USDA and Cooperative Programs

Problem Area		Scientist Man-Years FY 1969 (Estimated)		
No. :	Title and Activity	Intra- : mural	Extra- : mural	Total
407 :	New and Improved Feed, Textile and	:	:	:
:	Industrial Products from Field Crops	:	:	:
:		:	:	:
:	<u>Feed</u>	:	:	:
:	Microbiology and Toxicology	: 3.4	:	:
:	Total	: 3.4	:	:

Problems and Objectives

Tall fescue grass is grown extensively in the Southeast, in the Inter-mountain States, and in the Pacific Northwest as a forage crop for cattle and other domestic animals. It has excellent agronomic characteristics, producing well on marginal land and remaining green during cool weather when other grasses are dormant. Thirty-five to fifty million acres of fescue are grown for forage use in the southeastern part of the United States alone. Cattle grazing on pasture that is predominately tall fescue sometimes develop a disease known as "fescue foot." In severe attacks, animals become emaciated and frequently die. Elimination of this disease would prevent an estimated average economic loss of about \$5 million annually.

The major objectives of current research are to discover the cause of fescue foot disease and to develop and evaluate alternate ways for its prevention.

Progress - USDA and Cooperative Programs

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL
PRODUCTS FROM FIELD CROPS

Feed

A. Microbiology and Toxicology

1. Fescue toxicity. Fusarium tricinctum NRRL 3249, a mold previously isolated from toxic tall fescue pasture, produced the toxins 4-acetamido-4-hydroxy-2-butenic acid γ -lactone (I) and 8 α -(3-methylbutyryloxy)-4 β ,15-diacetoxyscirp-9-en-3 α -ol (II) when grown on laboratory media. Procedures were developed to obtain each mycotoxin in sufficient quantities for testing in young cattle. Intramuscular injection of subacute doses of compound I for 90 days led to a gangrenous tail, one sign of fescue toxicity, but did not lead to hoof lesions. Injection of compound II for 65 days led to a fatal hemorrhagic syndrome similar to that seen in the field when cattle consume moldy corn, from which another strain of F. tricinctum, NRRL 3299, was isolated. Of 146 fungal isolates from a toxic tall fescue pasture, almost all the molds producing metabolites toxic to mice belong to the genus Fusarium. Toxic Fusarium isolates were also obtained from a nearby orchard grass pasture. Orchard grass has not been implicated in cattle disease, perhaps because tall fescue is the predominant winter pasture in the climatic zone where fescue toxicity in cattle is found.

Several bacteria but no fungi were found to be inhibited by low concentrations of butenolide, a product of Fusarium tricinctum. Diacetoxyscirpenol, another toxic product of F. tricinctum, inhibits the growth of several yeasts and a mold but no bacteria. Extracts of toxic fescue hay inhibited the same bacterial strains as did the butenolide.

